PREPARATION AND CHARACTERIZATION OF PACLITAXEL NANOPARTICLE BY PRECIPITATION TECHNIQUE

Dissertation *Submitted to*

THE TAMIL NADU DR. M. G. R. MEDICAL UNIVERSITY,

CHENNAI – 600 032

In partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY IN

(PHARMACEUTICS)

Submitted By

ELAMIN IMADELDIN YOUSIF MOHAMED AHMED

261510001

Under the Guidance of

Dr. R. Kumaravelrajan, M.Pharm., Ph.D.,

(Institutional Guide)

D.Harinarayana, M.Pharm., Ph.D.,

(Industrial Guide)

DEPARTMENT OF PHARMACEUTICS

C.L.BAID METHA COLLEGE OF PHARMACY

(AN ISO 9001-2000 CERTIFIED INSTITUTE)

THORAIPAKKAM, CHENNAI- 600 097.

OCTOBER-2017

DECLARATION

I hereby declare that the thesis entitled **"PREPARATION AND CHARACTERIZATION OF PACLITAXEL NANOPARTICLE BY PRECIPITAION TECHNIQUE"** has been originally carried out by me under the supervision and guidance of **DR. R. Kumaravelrajan, M.Pharm., Ph.D.,** Associate Professor, Department of Pharmaceutics, C.L.BaidMetha College of Pharmacy, Chennai-97 during the academic year 2015-2016.

Date:

Place: Chennai-97

ELAMIN IMADELDIN YOUSIF MOHAMED AHMED
Department of Pharmaceutics
C.L.Baid Metha College of Pharmacy
Chennai- 97

ACKNOWLEDGMENT

Indeed, it is my great pleasure to express my humble gratitude on the successful completion of my thesis work. I am very thankful to my respective guide **DR. R. KumaravelRajan, M.Pharm., Ph.D.,** Associate Professor., Department of Pharmaceutics, C.L.BaidMetha College of Pharmacy, and Chennai – 97.

I am honored to work under his guidance and have precious guidelines, constant encouragement and support throughout my research work. I am very obliged for the patience with which he guided me at every step of my research work.

My special and sincere thanks to **DR. Grace Rathnam, M. Pharm., Ph.D., Principal and Head of the Department of Pharmaceutics** for continuous encouragement of this work.

I would like to thanks to our lab assistants **Mr. Rajaram, Mrs.Thenmozhiand Mr. Shankar** C.L.Baid Metha College of Pharmacy in helping me to utilize the lab facilities.

I also thanks to Store department in-charge **Mr. Srinivasan** C.L.BaidMetha College of Pharmacy for helping me to utilize the lab facilities and chemicals.

I specially thanks to all my seniors, classmates and juniors from the core of my heart for their help and encouragement.

Most significantly, I would like to express my grateful thanks to my parents for their endless love encouragement, blessing and motivation for the successful completion of my project.

(ELAMIN IMADELDIN YOUSIF MOHAMED AHMED)

Place:Chennai **[Reg. No:261510001]**

Date: **Dept. of Pharmaceutics**

Contents

List of Abbreviations

Abbreviation	Expansions
%	Percentage
^{o}C	Degree Centigrade
µg	Microgram
µm	Micrometre
mg	Milligram
ml	Millimetre
nm	Nano Meter
mm	Millimetre
cm	Centimetre
hr	Hour
NPs	Nanoparticles
CT	Colon targeting
BBB	Blood Brain Barrier
HPG	Hyper-branched Polyglycerol
MLV	Multilamellar vesicles
FTIR	Fourier Transform Infrared Spectroscopy

SEM	Scanning Electron Microscopy
IP	Pharmacopoeia of India
USP	United States Pharmacopoeia
ICH	International Conference on Harmonisation
NP	Nanoparticles
SUV	Small Unilamellar vesicles
LUV	Large Unilamellar Vesicles
K_e	Elimination rate constant
Log P	Partition coefficient
API	Active Pharmaceutical Ingredient
EE	Entrapment Efficiency
DL	Drug Loading
PS	Particle Size
Rpm	Revolution Per Min
RT	Room Temperature
SD	Standard Deviation
SM	Surface Morphology
UV	Ultra Violet

FDA	Food And Drug Administration
Avg.Wt	Average Weight
AUC	Area Under Curve
AUMC	Area Under First Moment Curve
$t_{1/2}$	Biological-half Life
Cmax	Peak Plasma Concentration
T^{max}	Time to peak concentration
Conc.	Concentartion
PX	Paclitaxel
USP	United States Pharmacopoeia
V_d	Volume of distribution
WHO	World Health Organisation
SLN	Solid Lipid Nanoparticles

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy

An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

ISO 9001 : 2008
Reg. No : RQ91/3038

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

DR. R. Kumaravelrajan, M.Pharm., Ph.D.,
Associate Professor

THE CERTIFICATE

This is to certify that the dissertation work entitled **"PREPARATION AND CHARACTERIZATION OF PACLITAXEL NANOPARTICLE BY PRECIPITAION TECHNIQUE"** submitted to **THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY, CHENNAI-32** for the award of the degree of **Master of Pharmacy in Pharmaceutics** is a bonafide research work done by **Reg. No. 261510001** under my guidance in the **Department of Pharmaceutics**, C.L.BaidMetha College of Pharmacy, Chennai-600097 during the academic year 2016-2017.

Place: Chennai-97

Date:

(DR. R. Kumaravelrajan)

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy

An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

ISO 9001 : 2008
Reg. No : RQ91/3038

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

Prof. DR. (Mrs). Grace Rathnam, M.Pharm. Ph.D.,

HOD & Principal,

THE CERTIFICATE

This is to certify that the dissertation work entitled **"PREPARATION AND CHARACTERIZATION OF PACLITAXEL NANOPARTICLE BY PRECIPITAION TECHNIQUE" submitted** to **THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY, CHENNAI- 32** for the award of the degree of Master of Pharmacy in Pharmaceutics is a bonafide research work done by **Reg. No. 261510001** under the guidance of **DR. R. Kumaravelrajan, M.Pharm., Ph.D., Associate Professor, Department of Pharmaceutics,** C.L.BaidMetha College of Pharmacy, Chennai-600097 during the academic year 2016-2017.

Place: Chennai-97

Date:

DR. Grace Rathnam, M.Pharm.Ph.D.,

HOD &Principal
Department of Pharmaceutics
C.L.BaidMetha College of Pharmacy
Chennai- 600097

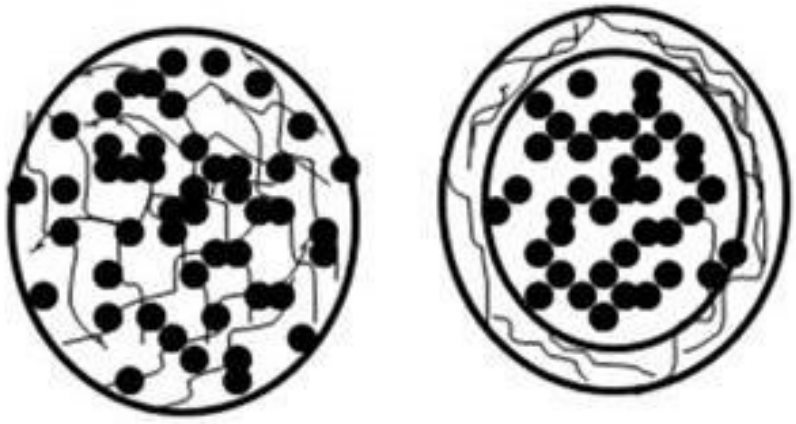
Introduction

Nanoparticles play a very important role in cancer research. Due to extremely small size of Nanoparticles they are easily and more readily taken up by the human body. Biological membranes and access cells, tissues and organs are eligible for entrance of Nanoparticles. Nanoparticles are stable, solid colloidal particles consist of biodegradable polymer or lipids and size range 10-1000 nm. Nanoparticles have attracted the attention of scientists because of their multifunctional character. Nanoparticles have greater surface area to volume ratio, that helps in the diffusion process. Nanoparticles also leading to special properties such as increased heat and chemical resistance. A single cancerous cell, giving a strain on the nutrient supply and removal of metabolic waste products. If small tumor has formed, the normal tissue will not be able to oppose the cancer cells for the normal supply of nutrients from the blood.[1]

The advantages of Nanoparticles as drug delivery systems include time controlled drug delivery, reduced drug toxicity, improved bioavailability and enhanced therapeutic efficacy and biodistribution.[2] Nanoparticles can also protect the sensitive drugs from degradation by environmental factors such as stomach acid and enzymes.[3] PolymericNanoparticles range in size from about 10-1000 nm, and can be modified with different ligands such as antibodies to create a smart targeting delivery system.[4] Polymeric Nanoparticles of a size around or less than 300 nm coated with surfactants have been proved to be able to transport drugs across the Blood Brain Barrier BBB. [5]

Nano-Capsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane .

Nanoarticles are matrix systems in which the drug is physically and uniformly dispersed.(**Fig 1).**



a b

Fig.1: Schematic Representation of Nanoparticles in matrix system (a) , Nanocapsules in polymer membrane system (b)

In recent years, biodegradable Polymeric Nanoparticles, particularly those coated with hydrophilic polymer such as Poly(ethylene Glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver **(Fig 2).**

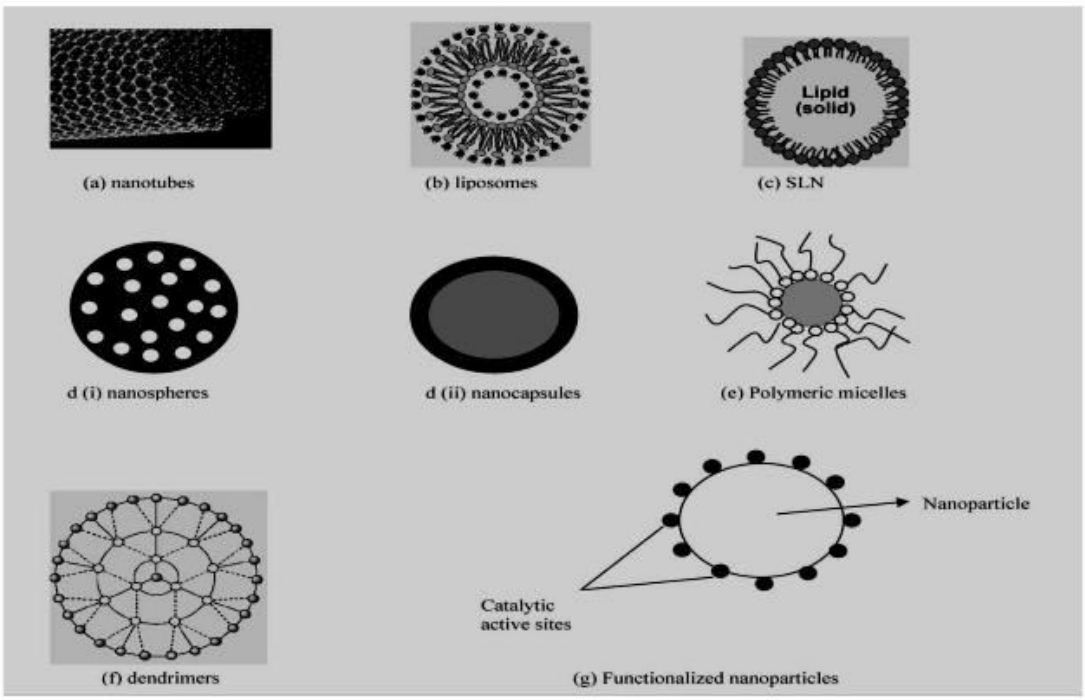


Fig.2: Nanoparticle Drug Delivery System

1.1 Etiology of Cancer

The colon is the part of the digestive system where the waste material is stored. The rectum is the end of the colon adjacent to the anus. Together, they form a long, muscular tube called the large intestine (also known as the large bowel). Tumors of the colon and rectum are growths arising from the inner wall of the large intestine. Benign tumors of the large intestine are called polyps **(Fig 3)**. Malignant tumors of the large intestine are called Cancers. Benign polyps do not invade nearby tissue or spread to other parts of the body. Benign polyps can be easily removed during colonoscopy and are not life-threatening. If benign polyps are not removed from the large intestine, they can become malignant (cancerous) over time. Most of the cancers of the large intestine are believed to have developed from polyps. Cancer of the colon and rectum (also referred to as colorectal cancer) can invade and damage adjacent tissues and organs. Cancer cells can also break away and spread to other parts of the body (such as liver and lung) where new tumors form. The spread of colon cancer to distant organs is called metastasis of the colon cancer **(Fig 4)** and **(Fig 5)** Once metastasis has occurred in colorectal cancer, a complete cure of the cancer is unlikely.

Globally, cancer of the colon and rectum is the third leading cause of cancer in males and the fourth leading cause of cancer in females

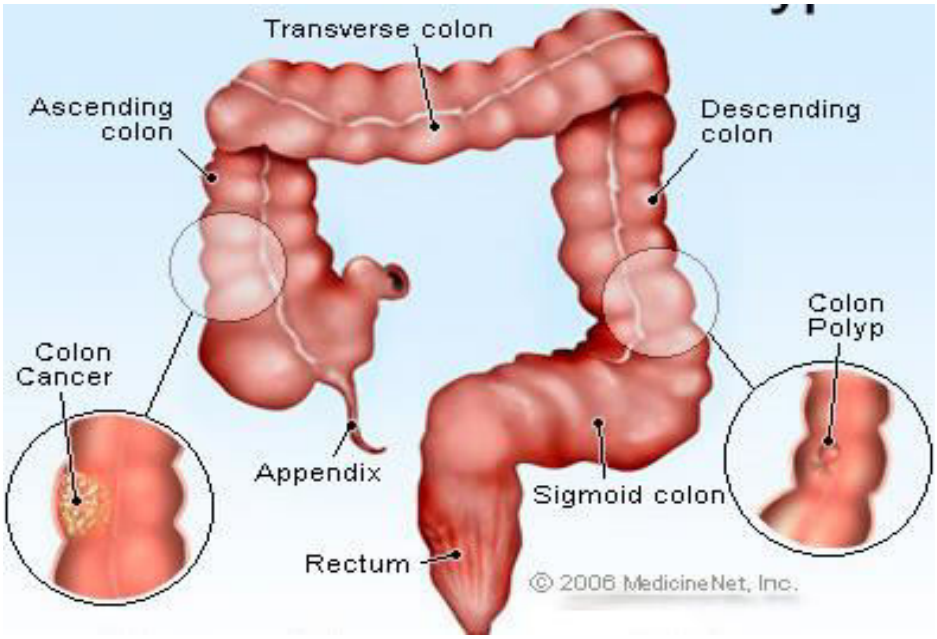


Fig.3:Diagrammatic representation of colon cancer

Fig.4: Micrograph of a tubular adenoma (left of image), a type of colonic polyps anda precursor of colorectal cancer.

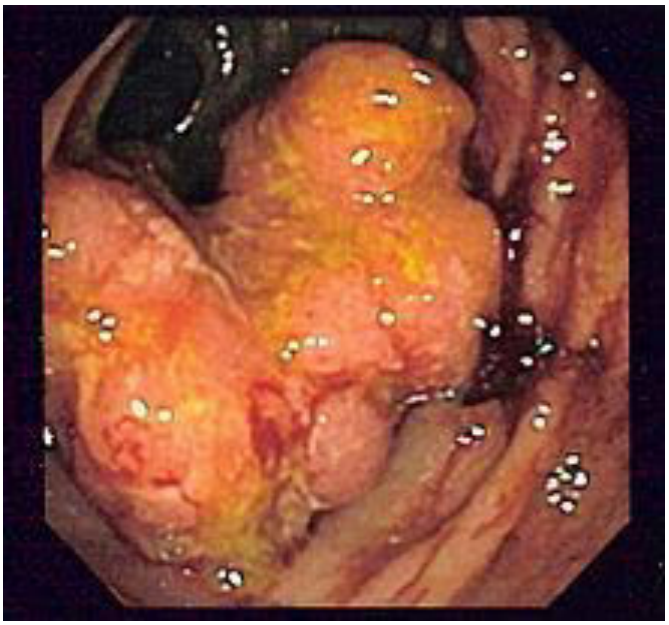


Fig.5: Endoscopic image of colon cancer identified in sigmoid colon on screening.

Cancer therapy strategies are currently focussed on surgery, chemotherapy, radiotherapy, immunotherapy and hormonal therapy. These conventional strategies are limited by the accessibility to the tumor and the lack of selectivity towards tumor cells, the spread of cancer cells throughout the body, and the risk of operating on a vital organ. Regarding cancer chemotherapy, treatment failure is frequently encountered even in the most sensitive cancers to chemotherapy agents.[6] Several reasons have been pointed out for chemotherapy failure: i) the Physicochemical properties of many drugs, e.g., hydrophobicity, promotes the unsuccessful localization at the cancer site ii) Unfavorable pharmacokinetics (rapid clearance and rapid *in vivo* degradation) determine the need of higher doses and rigorous treatment schedules to obtain a therapeutic effect iii) The relative poor selectivity of chemotherapy agents for targeted tumor cells iv) the large biodistribution and non-intended extravasation with severe side effects in non-targeted sites; and v) The susceptibility to induce drug resistance.[7, 8, 9].

Cancer physiology is also responsible for the chemotherapy failure, mainly because of the absence of a non-functional lymphatic system that allows drug

escaping out of the tumor, and due to a very high hydrostatic pressure gradient inside the tumor that difficult a uniform drug diffusion inside the tumor.[10,11] The association of anti-tumor drugs to colloidal delivery systems in cancer treatment has been proposed to improve their efficacy and to reduce their associated toxicity. This strategy could allow obtaining a specific accumulation at the tumor site, an improvement of the pharmacokinetic profile, a prolongation of the exposure of the tumor cells to these active agents and a minimization of the severe side effects.[7,12]

With this, it have been established that a suitable anti-tumor drug delivery system should have the following properties: i) small size (≤ 500 nm) to allow a large biodistribution and an adequate perfusion at the target site ii) the ability to deliver therapeutic drug quantities, without overloading the organism with foreign material; iii) physical stability and low drug leakage problems under storage and *in vivo* iv) controlled drug release rates exclusively at the targeted tumor; and v) maximum biocompatibility and biodegradability (with very low toxicity of breakdown products), and minimal antigenicity.[7,13] These drug carriers are frequently based on vesicular (liposomes and niosomes) and polymeric systems. Special approaches such as surface-functionalization (e.g., with specific ligands to tumor cells) and engineering of stimuli-sensitive materials, could enhance the biodistribution profile of loaded drugs and, thus, resulted in a more efficient tumor therapy.[7,11,14]

One of the most promising materials for the design of nanocarriers loaded with chemotherapy agents is the biodegradable polymer poly(ε-Caprolactone) (PCL). This aliphatic polyester is very suitable for controlled drug delivery due to its high permeability to many drugs and non-toxicity, its exceptional ability to form blends with other polymers, and its very low degradation rate (compared to other well known drug carriers, such as Poly(D,Lactide-co-glycolide) (PLGA).[15]

Table 1:Types of Nanoparticles applied in Drug Delivery Release System

S.No	Type of Nanoparticle	Materials Used	Application
1	**Nanosuspensions and Nanocrystals**	Drug powder is dispersed in surfactant solution	Stable system for controlling delivery of poorly soluble drug
2	**Solid lipid Nanoparticles**	Melted lipid dispersed in Aqueous surfactant	Least toxic and more stable Colloidal carrier systems as alternative materials For polymers
3	**Polymeric Nanoparticles**	Biodegradable polymers	Controlled and targeted Drug delivery
4	**Polymeric micelles**	Amphiphilic block Co polymers	Controlled and systemic Delivery of water insoluble Drugs
5	**Magnetic Nanoparticles**	Magnetite Fe_2O_3, Meghe Mite coated with dextran	Drug targeting diagnostics to in medicine
6	**Carbon Nanotubes**	Metals, semiconductors or carbon	Gene and DNA delivery Controlled release of drugs
7	**Liposomes**	Phoshpolipid vesicles	Controlled targeted drug Delivery
8	**Nanoshells**	Dielectric core and metal shell	Tumor targeting
9	**Ceramic Nanoparticles**	Silica, alumina, Titania	Drug and biomolecule Delivery
10	**Nanopores**	Aerogel, which is produced by cell gel chemistry	Controlled release drug Carriers
11	**Nano wires**	Silicon, cobalt, gold or Copper based nanowires	Electron transport in nano Electronics
12	**Quantum dots**	CD Se-CDs core shell	Targeting, imaging agent
13	**Nano films**	Polypeptides	Systemic or local drug Delivery.
14	**Ferrofluids**	Iron oxide magnetic Nanoparticles surrounded by polymeric layer.	For capturing cells and other biological targets.

1.2.1 Nanosuspension

A suspension of drug Nanoparticles in a liquid is called as Nanosuspension. A size of the Nanoparticle lies in between 200 to 500 nm. and exceptional feature of nanosuspension is the increased saturation, solubility, the increased dissolution rate of

compound.[16] The saturation and solubility increase below a particle size of 1 mm. An additional characteristic of nanosuspension is that they may induce changes in the crystalline structure increases the amorphous portion in particle or even creating completely amorphous particles. Nanoparticles and Nanosuspensions show an increased adhesiveness to tissue. The oral administration of drugs in the form of Nanosuspension

Examples of Nanosuspension: Nanosuspension of Ibuprofen is prepared by emulsion-solvent diffusion technique for the purpose of improving ocular availability, whereas nanosuspension of Danazole is formulated by nanocrystal technology to improve bioavailability.[17]

1.2.2 Solid Lipid Nanoparticles (SLN)

The solid lipid Nanoparticles are sub micron colloidal carriers (50-1,000nm) which are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. In order to overcome the disadvantages associated with liquid state of oil droplets, liquid lipid replaced by a solid lipid, which eventually transformed into solid lipid Nanoparticles.[18]

1.2.3 Polymeric Nanoparticles

The drug is dissolved, entrapped, absorbed, attached or encapsulated into Nanoparticle matrix. Depending on the process of preparation, Nanoparticles, Nanospheres or Nanocapsules can be obtained with different properties and release characteristics for an encapsulated therapeutic agent.Nanoparticles are vesicular systems in which the drug is restricted to a cavity surrounded by unique polymer

membranes, whereas nanospheres are matrix systems in which the drug is physically and uniformly dispersed. The advantages of using Nanoparticles for drug delivery result of their two main basic properties. First, Nanoparticles, because of their small size, can penetrate through smaller capillaries and are taken up by cells, which allow efficient drug accumulation at the target sites. Second, the use of biodegradable materials for Nanoparticle preparation allows sustained drug release within the target site over a period of days or even weeks.[19]

1.2.4 Polymeric Micelles

Polymeric micelles have been extensively studied as drug carriers. Polymeric micelles have enhanced thermodynamic stability in physiological solution, as indicated by their low critical micellar concentration, which makes polymeric micelles stable and avoid their rapid dissociation *in vivo*.

Micelles have a fairly narrow size distribution in the nanometer range and are characterized by their exclusive core-shell architecture, in which hydrophobic segments are segregated from the aqueous exterior.

Micellar systems are useful for the systemic delivery of water-insoluble drugs. Drugs can be partitioned in the hydrophobic cores of micelles and the outer hydrophilic layer from steady dispersion in aqueous media which can then be administered intravenously. The distribution of drug-loaded polymeric micelles (less than 100 nm in diameter), following intravenous administration, polymeric micelles have been shown to have extended systemic circulation time because of their smaller size and hydrophilic shell, which minimizes their uptakes by the reticuloendothelial System. Polymeric micelle-incorporated drugs may accumulate to a greater extent than free drugs into tumors and exhibit reduced distribution in nontargeted areas.[20,21]

1.2.5 Magnetic Nanoparticles

Magnetic Nanoparticles are influential and adaptable diagnostic device in the field of medicine. Magnetic immunoassay techniques have been developed in which the main ground generated by the magnetically labeled target detected directly with sensitive magnetometer. Superparamagnetic Nanoparticles are used as contrast agents in magnetic resonance imaging .The magnetic Nanoparticle are coated with inorganic core of iron oxide with polymer such as Dextran. Magnetic Nanoparticles of Indomethacin demonstrated selective targeting under magnetic field of 8000 OE-strength, following normal administration, the drug concentration was higher in the Liver and Spleen where endocytosis and phagocytosis could occur.[22]

1.2.6 Carbon Nanotubes

Carbon Nanotubes are a new form of carbon molecule around in a hexagonal complex of carbon atoms, these hollow cylinders can have diameter as a small as 0.7nm and reach several millimeters in length. Each end can be opened or closed by a fallen half molecule. The small dimensions of Nanotubes, combined with their remarkable physical, mechanical and electrical properties, make them unique materials. The mechanical strength of carbon Nanotubes is more than sixty times greater than that of the best steel, even though they weigh six times less. They also represent a very large specific surface area, are outstanding heat conductors and display unique electronic properties, offering three dimensional configurations. They have higher capability for molecular absorption.[23]

1.2.7 Liposomes

Liposomes have been used as a versatile tool in biology, biochemistry and medicine. Liposomes are small synthetic vesicles of spherical shape that can be produced from natural, non toxic Phospholipids and Cholesterol. Because of their size, hydrophilic and hydrophobic character, as well as biocompatibility, liposomes is

promising system for drug delivery. Properties of Liposomes are different substantially with lipid composition, size, surface charge and the method of preparation. They are therefore classified into three classes based on their size and number of bilayers. Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25-50nm in diameter. Large Unilamellar Vesicles (LUV) are a heterogeneous group of vesicles similar to SUVs and are surrounded by a single lipid layer. Multilamellar vesicles (MLV) consist of several lipids separated from one another by a layer of aqueous solution.Drugs associated with liposomes have markedly altered pharmacokinetics properties compared to drugs in solution. They are also effective in reducing systemic toxicity and preventing early degradation of the encapsulated drug after introduction to the target organism.[24]

1.2.8 Nanoshells coated with Gold

Gold nanoshells are new composite Nanoparticles that combine infrared optical activity with the uniquely biocompatible properties of gold colloid. Metal nanoshells are concentric spherical Nanoparticles consisting of a dielectric (typically Gold sulfide or silica) core and a metal (Gold) shell. By varying the relative thickness of the core and shell layers, the plasmon-derived optical resonance of gold can be considerably shifted in wavelength from the visible region of highest physiological transmissivity. By varying the absolute size of the gold nanoshell, it can be made to either selectively absorb (for particle diameter < 75nm) or scatter incident light. Because the gold shell layer is deposited using the same chemical method used to grow gold colloid, the surface properties of gold nanoshells are nearly identical to those of gold colloid. Gold Nanoshells can be used to breast cancer cells.[25]

1.2.9 Ceramic Nanoparticles

The newly rising area of using inorganic (ceramic) particles with entrapped biomolecule has possible applications in many frontiers of modern materials science including drug delivery system. The advantages of ceramic Nanoparticles include

easy preparation with the desired size, shape and porosity, and no effect on swelling or porosity with no change in the.[26]

1.2.10 Nanopores

Materials with defined pore-sizes in the nanometer range are of special interest to a broad range of industrial application because of their wonderful properties with regard to thermal insulation, controllable material separation and release and their applicability as templates or fillers for chemistry and catalysis. One example of nonporous material is aerogel, which is produced by soil-gel chemistry.[26]

1.2.11 Nanowires

Nanowires are conductive or semi conductive particles with a crystalline structure of a few dozen nm and a high length /diameter ratio. Silicon, Cobalt, Gold or Copper-based nanowires have already been produced. They are used to transport electrons in nanoelectronics they could be composed of different metals, Oxides, Sulfides and Nitrites.[27]

1.3 Benefits of Nanoparticles drug delivery system

- Nanoparticles can easily pass through the smallest capillary vessels due to their ultra tiny volume.
- They can avoid the rapid clearance by phagocytes so that the duration in the bloodstream can be prolonged.
- Nanoparticles can easily penetrate the cells and tissue gap to arrive at target organs eg. Liver, Spleen, Sungs, spinal cord, and Lymph's.
- It shows the controlled release property.
- Site specific targeting by attaching the ligands to the surface of the spheres.
- They can be easily administered by various routes including oral, nasal, parenteral, etc.

- Reduction of toxicity is also an important advantage of Nanospheres.

1.4 Drawback of Nanoparticles drug delivery system

- The physical handling of Nanospheres is difficult in liquids and in dry form.

  Due to the smaller size and larger surface area of Nanospheres, chances of particle aggregation increases.

- Drug loading and burst release are limited due to the smaller size and larger surface area.[28]

1.5 Nanoparticles as targeted drug delivery

There are various ways of using of Nanoparticles as targeted drug delivery system.

1.5.1 Targeting of the tumor

Basically Nanoparticles are able to distribute the concentrate dose of the drug to the tumor targets through permeability enhancing and retention effect or active targeting by ligands on the surface of Nanoparticles. It Is can decrease the toxicity by reducing the drug disclosure of health tissue by limiting drug distribution to the target organ. Nanoparticles have a higher concentration manifested in Liver, Spleen, Lungs than in other parts of the body.By this study we can say that there is no doubt that Nanoparticles have an effective role in the treatment of cancer. But they have a drawback which was reported that during distribution of drug which is incorporated into the Nanoparticles mainly accumulated in the liver. 56% of drug accumulated in the liver and only 1.6% of drug reaches the tumor. Thus we can say that Nanoparticles have a greater tendency to be captured by the liver. So it is a great challenge to avoid particle uptake by the Mononuclear Phagocyte System (MPS) in spleen and liver for using Nanoparticles for tumor targeting. It has been proved that using doxorubicin with Nanoparticles have a great effect against hepatic metastasis than free drug used.

1.5.2 Long circulation of Nanoparticles

Basically Nanoarticles are able to target tumors, which are localized outside MPS For long circulation of Nanoparticles a major break came in the field when hydrophilic polymer (PEG, Poloxamine) is coated on the surface of Nanoparticles by which reverse effect is produced to the uptake by the MPS.

The coating provides a cloud of hydrophilic and neutral chain at the particle surface which repels plasma proteins. As a result coated Nanoparticles become imperceptible to MPS and remain for a longer duration during circulation.

1.5.3 Nanoparticles for oral delivery

It is very hard to use the bioactive molecules (peptides and proteins) with suitable carriers. These suitable carriers remain a challenge due to the fact that bioavailability of these molecules is limited and they get degraded by enzymatic action. So the polymeric Nanoparticles permit encapsulation of bioactive molecules and protecting them against enzymatic degradation.

1.5.4 Nanoparticles for drug delivery in the brain

In central nervous system the most important factor is Blood brain barrier (BBB) for the growth of new drugs and it is characterized by impervious endothelial cells with tight junction, enzyme activity and active transport systems.

Basically the BBB only permits selective transport of molecules. So if we use Nan oparticles as targeted drug delivery it will act together with specific receptor-mediated transport system in BBB. E.g. Polysorbate 80/LDL is capable of delivering. So the drugs which cannot easily cross the BBB, can pass easily with the help of nanoparticles.

There are also other drug delivery systems present for this purpose.

- Nanoparticles for gene delivery
- Nanoparticles targeting of epithelial cells etc.[29]

Table 2:**U.S Patent for Paclitaxel Anti-Cancer Drug**

S.No	Patent No	Type	Year
1.	**U.S. Pat. No. 5,641,803, U.S. Pat. No. 5,670,537**	Paclitaxel based anti-tumor for mulation	**Jan 25, 2007**
2.	**Pat. No. 5,439,686, U.S. Pat. No. 5,498,421, U.S. Pat. No. 5560933**	Paclitaxel based anti-tumor formulation	**Oct2. 2003**
3.	**U.S. Pat. No. 5,439,686, U.S. Pat. No. 5,498,421, U.S. Pat. No. 5,560,933**	Process of producing Nanoparticles and albumin	**Jun 8, 2006**
4.	**U.S.Pat.Nos.5,916,596;6,506,405; 6,749,868**	Nanoparticle Compositions of albumin and Paclitaxel	**Jul3. 2014**

Review of Literature

Mamun Ur Rashid *et al* (2013).,[30] developed Silver Nanoparticles (Ag-NPs) prepared by using chemical synthesis. Silver nanocolloid solution has been prepared chemically by the Precipitation of silver salt using sodium borohydride ($NaBH_4$) and trisodium citrate ($C_6H_5Na_3O_7.2H_2O$). The Nanoparticles were characterized by UV-VIS spectrophotometry and Scanning Electron Microscopy (SEM).

Yujie Wang *et al* (2002).,[31] studied Paclitaxel-loaded Nanoparticles incorporated with PVLA were prepared by emulsion solvent evaporation method with polyvinyl alcohol (PVA) as co-emulsifier. The presence of PVLA on the particle surface was investigated through the change of Zeta potential and surface hydrophobicity. Cellular uptake and cytotoxic activity, involving factors concerned with them, were evaluated by Hep G2 cells *in vitro*.

Ngoc Duong Trinh *et al* (2015).,[32] prepared Silver chloride Nanoparticles by the precipitation reaction between silver nitrate and sodium chloride in an aqueous solution containing poly (vinyl alcohol) as a stabilizing agent. Different characteristics of the Nanoparticles in suspension and in lyophilized powder such as size, morphology, chemical nature, interaction with stabilizing agent and photo-stability were investigated. Biological tests showed that the obtained silver chloride Nanoparticles displayedantibacterial activities against *Escherichia coli* and *Staphylococcus aureus*.

Dinda *et al* (2013).,[33] Formulated Paclitaxel loaded SLNs prepared by Solvent emulsification and evaporation method using ultra sonication and optimization of critical process variables were carried out to develop stable SLNs. The average particle size of SLNs was found to be 63 nm ± 5.77 with Poly dispersity index (PDI) 0.272 ± 0.02 and entrapment efficiency was

found 94.58%. The stability studies and zeta potential were performed at refrigerated temperature (2-8°C) indicating no significant increase in particle size after one month storage. *In-vitro* release studies showed initial burst release followed by controlled release for 48hrs (about 73%).

Shu-Ben Sun *et al* (2015).,[34] prepared Capecitabine NPs were characterized by FT-IR, DSC, drug Loading, entrapment efficiency, particle size, surface morphology by Atomic Force Microscopy (AFM), X-ray diffraction and *in-vitro* studies. FT-IR and DSC studies indicated that there was no interaction between the drug and polymer. The morphological studies performed by AFM showed uniform and spherical shaped discrete particles without aggregation and smooth in surface morphology with a nano size range of 144 nm. X-ray diffraction was performed to reveal the crystalline nature of the drug after encapsulation.

Natarajan Tamilselvan *et al* (2015).,[35] Formulated anti-alzheimers drug loaded chitosan Nanoparticles by simple ionic gelation method using various concentrations of chitosan and TPP. The prepared Nanoparticles were evaluated for particle size, shape, charge, encapsulation efficiency, *in vitro* drug release and *in vitro* cytotoxicity. Results: The optimized drug loaded Nanoparticles showed the size of 125 ± 4mn with PDI 0.25 ± 0.05, potential of +40 ± 2mV, encapsulation efficiency of 65.5 ± 1.2% and the drug release of 68.4 ± 1.6% with an initial burst effect up to one hour followed by sustained release up to 24 hrs. Further the optimized formulation was subjected to investigate the cytotoxicity of CS-NP in SH-SY-5Y cell lines it revealed that the cell viability was above 90% without any toxicity.

CA Anuradha *et al* (2010).,[36] has studied Bis-demethoxy Curcumin Analogue (BDMCA) Nanoparticle formulations were fabricated by a double emulsion solvent evaporation technique using polycaprolactone as the polymer. The Nanoparticles were characterised for drug content, particles size, *in vitro* drug release and the drug-polymer interaction. The *in vivo*

properties of the formulations in male Wistar rats were evaluated from the pharmacokinetics and pharmacodynamics of BDMCA following i.v. administration of the Nanoparticles. BDMCA solution was administered i.v. as a reference. Hepatoprotectivity of the formulation was determined in treated rat model.

Jiaojiao Xu *et al* (2015).,[37] developed Nanoparticles for Chronic intracellular infections caused by drug-resistant Pathogens pose a challenge to the treatment of chronic osteomyelitis is. The results showed that the prepared Cefixime Nanoparticles were predominantly spherical in shape with an average par ticle diameter of 220 nm, a positive zeta potential, and a loading efficiency of 73.65% ˘1.83%. Furthermore, their drug release profile followed the Higuchi model for sustained release, with non-Fickian diffusion.

Shanmuganathan Seetharaman *et al* (2016).,[38] prepared Cefixime Nanoparticles by the modified-coacervation method. The prepared drug Nanoparticles were evaluated for particle size, zeta potential, surface morphology, entrapment efficiency, *in-vitro* drug release studies and also in-vitro antimicrobial efficiency studies for the selected ideal batch. The surface morphology of the prepared Nanoparticles was found to be spherical with smooth surface and entrapment efficiency was found to be 70.4±1.2 to 83.4±2.0. The *in-vitro* drug release showed a biphasic pattern with initial burst release followed by the sustained release of the drug up to 24h.

YI Yi-Mu *et al* (2015).,[39] prepared 5-FU sodium alginate bovine serum albumin Nanoparticles by Emulsion solidification method, and to determine its diameter under Transmission Electronic Microscope (TEM). Then the rate of NP and external drug releasing velocity were measured. The average arithmatic diameter of the NP was 166nm±34nm, the rate of 5-FU was 32.8% and the cumulative external releasing ratio amounted to 84.0% within 72

hours. The NP was mainly distributed in the liver, spleen, lungs and kidneys after NP oral administration to rats.

Shashank Tummala *et al* (2015).,[40] prepared Carbamazepine NP. The polymeric Nanoparticles were subjected to particle size evaluation, SEM study, drug content, entrapment efficiency and *in vitro* release studies. Nanoparticles with drug: polymer ratio of 1:1 has shown a particle size, drug loading and entrapment efficiency of 130±2.1nm, 61.28% and 18.15% respectively. Optimized batch in drug: polymer ratio of 1:1 has shown a particle size, drug loading and entrapment efficiency Contour plots and 3D-scatter plots were drawn for statistical supportive evaluation in optimization using Minitab 17.

Pragati Lingwal *et al* (2015).,[41] prepared Ampicillin Nanoparticles by Desolvation method and characterized for drug content, particle size and size distribution, zeta potential, and *in vitro* drug-release study. In this method the Bovine Serum Albumin Nanoparticles were prepared by Desolvation technique. Ampicillin belonging to the Penicillin group of beta-lactam antibiotics. It is used for the treatment of infections known to be or highly likely to be caused by bacteria. All formulations were further checked for evaluation parameters. Particle size of all formulation was found to be 200 to 500nm. Zeta potential for all formulated Nanoparticles were in the range of 64.1which indicates excellent stability.

Vyjayanthimala *et al* (2014).,[42] studied Nanoparticles of Zidovudine using chitosan, liquid paraffin and Tween-20 using Emulsion droplet coalescence method. The concentration of the polymer Chitosan was selected based on the results on preliminary screening. The Nanoparticles prepared were evaluated for morphology, loading efficiency and *in vitro* release. The particle shape and morphology of the prepared Zidovudine Nanoparticles

were determined by SEM analysis. The amount of Zidovudine entrapment in the Nanoparticles was calculated by the difference between the total amount of drug added to the Nanoparticle and the amount of non-entrapped drug remaining in the aqueous supernatant. A Franz diffusion cell.

Shumaia Parvin *et al* (2014).,[43] SLNPs are prepared by hot homogenization method at different ratio of drug, lipid, surfactant and stabilizer and designated as DNP1 to DNP6. *In vitro* dissolution study was performed using the USP type II apparatus (paddle method) at 50 rpm to a temperature of 37°±0.5°C in distilled water containing 0.75% w/v SLS (sodium lauryl sulfate). The absorbance of sample was measured spectrophotometrically at λmax 239nm on a UV-Visible spectrophotometer. Release pattern of drug was found to follow zero order, first order and Korsmeyer-Peppas equations.

Anand Kumar Kushwaha *et al* (2013).,[44] prepared Raloxifene loaded SLN by solvent emulsification/evaporation method, and different concentrations of surfactant, and homogenization speed were taken as process variables for optimization. SLN were characterized for particle size, zeta potential, entrapment efficiency, surface morphology, and crystallinity of lipid and drug. *In vitro* drug release studies were performed in phosphate buffer of pH 6.8 using dialysis bag diffusion technique. Particle sizes of all the formulations were in the range of 250 to 1406 nm, and the entrapment efficiency ranges from 55 to 66%. FTIR and DSC studies indicated no interaction between drug and lipid, and the XRD spectrum showed that RL-HCL is in amorphous form in the formulation.

Riddhi Dave *et al* (2012).,[45] prepared and evaluated Chitosan Nanoparticles containing Doxorubicin by the w/o emulsion method. SEM indicated spherical structure of Nanoparticle without agglomeration. *In vitro* drug release study suggests sustain drug release for a longer period of time. In particle size analysis, it was found to be in nano range 210 nm which shows

the formation of Nanoparticle with uniform size distribution. The drug entrapment was found to be 53.12% in optimized batch. The release shows that the drug entrapped within the polymeric matrix and it follows peppas diffusion controlled mode.

Can Zhanga *et al* (2008).,[46] used Bovine serum albumin (BSA) to prepare the microspheres loading with 5-Fluorouracil (5-FU) by means of chemical crosslinking method and then the microspheres were coated with N-galactosylated chitosan by electrostatic interaction. The structure of coating layers on the surface of 5-FU-loaded BSA microspheres was characterized by attenuated total reflection Fourier (ATR-FTIR), electron spectroscopy for chemical analysis (ESCA), wide X-ray diffraction (WXRD) and transmission electron microscopy (TEM). The properties of the coated microspheres containing 5-FU were determined.

C. Zinuttia *et al* (2003).,[47] developed Ethylcellulose microspheres containing 5-fluorouracil (5-FU) by a solvent evaporation technique using light mineral oil as the continuous phase. The drug was suspended in the acetone solution of the polymer. Three drug/polymer ratios (1/1, 1/2 and 1/3) were utilized. The microspheres were studied with respect to size, drug content and surface characteristics; the higher the polymer content, the smoother the microspheres. The drug was suspended in the polymer and the drug loading was important (more than 90%) with the three types of microspheres.

Drug Profile

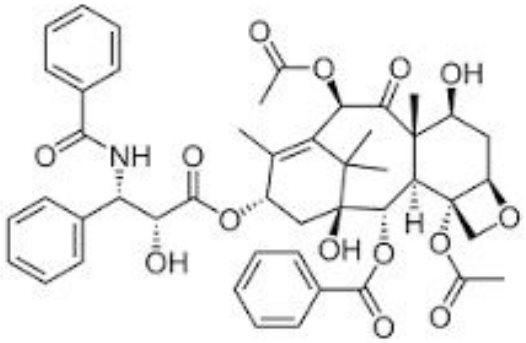
3.1 Drug Profile[48, 49, 50]

3.1.1 Description

Paclitaxel is a compound extracted from the Pacific yew tree Taxus brevifolia with antineoplastic activity. Paclitaxel binds to tubulin and inhibits the disassembly of microtubules, thereby resulting in the inhibition of cell division. This agent also induces apoptosis by binding to and blocking the function of the apoptosis inhibitor protein Bcl-2 (B-cell Leukemia 2). (NCI04).

Source: MeSH

Record Name: Paclitaxel



Chemical name - Paclitaxel; TAXOL; Taxol A; Abraxane; Paxene; Paxceed

Molecular formula - $C_{47}H_{51}NO_{14}$

Molecular weight - 853.91g/mol

Category - Antimicrotubule agent

Physical and chemical properties

Colour - White to off-white crystalline powder

Form - Fine white powder

Odour - Odourless

State	-	Solid
pKa	-	10.36
Melting temperature	-	216-217 °C
LogP	-	3
Log s	-	-5.2

3.1.2 Solubility

Soluble in methanol (50 mg/ml - clear, colorless solution), DMSO (50 mg/ml - clear, colorless solution), chloroform and ethanol. DMSO solutions can be stored aliquoted and frozen at -20°C for several months. Paclitaxel undergoes transesterification in methanol, and it hydrolyzes in aqueous solutions; Hardly soluble in water.

3.1.3 Mechanism of action

Paclitaxel interferes with the normal function of microtubule growth. Whereas drugs like colchicine cause the depolymerization of microtubules *in vivo*, paclitaxel arrests their function by having the opposite effect; it hyper-stabilizes their structure. This destroys the cell's ability to use its cytoskeleton in a flexible manner. Specifically, paclitaxel binds to the β subunit of tubulin. Tubulin is the "building block" of mictotubules, and the binding of paclitaxel locks these building blocks in place. The resulting microtubule/paclitaxel complex does not have the ability to disassemble. This adversely affects cell function because the shortening and lengthening of microtubules (termed dynamic instability) is necessary for their function as a transportation highway for the cell. Chromosomes, for example, rely upon this property of microtubules during mitosis. Further research has indicated that paclitaxel induces programmed cell death (apoptosis) in cancer cells by binding to an apoptosis stopping protein called Bcl-2 (B-cell leukemia 2) and thus arresting its function.

3.1.4 Pharmacokinetics (ADME):

Paclitaxel is a taxane. Paclitaxel binds to tubulin, the protein component of microtubules, simultaneously promoting their assembly and disassembly to form stable, nonfunctional microtubules. Although some reports indicate a cross-reactivity rate of 90% between docetaxel and paclitaxel, others suggest it does not occur consistently. Stabilization of microtubules blocks cells in the M phase of the cell cycle, inhibiting cell division and causing cell death. Paclitaxel acts as a radiosensitizing agent by blocking cells in the G2phase. 4 Paclitaxel is an immunosuppressant.

3.1.5 Adverse Effects

Emetogenic potential **:** Low

Extravasation Potential **:** Irritant

Table 3: Side Effect of Paclitaxel Nanoparticles

ORGAN SITE	SIDE EFFECT
Clinically important side effects are in ***bold, italics***	
cardiac	bradycardia (3-4%); first 3 h of infusion ; see paragraph following **Side Effects** table
	cardiovascular events (severe 1-2%) , see paragraph following **Side Effects** table
ear and labyrinth	hearing loss, tinnitus, vertigo, ototoxicity (<1%)
eye	optic nerve and/or visual disturbances, photopsia, visual floaters (<1%); generally reversible, may be dose-related
gastrointestinal	*emetogenic potential: low-moderate*
	abdominal pain; with intraperitoneal administration
	anorexia (25%)
	constipation (18%)
	diarrhea (25-79%)
	intestinal obstruction (4%)
	mucositis (20-31%); more common with 24 h infusion
	nausea and vomiting (44-52%)
	taste changes
general disorders and administration site conditions	*extravasation hazard: irritant, treat as vesicant* , see paragraph following **Side Effects** table
	edema (17-21%, severe 1%); localized under skin at no specific site
	fever (12%)
	injection site reactions (4-13%)
immune system	***hypersensitivity reactions*** (5-42%, severe 1-2%) ; see paragraph following **Side Effects** table
infections and infestations	infections (18-30%, severe 1%); primarily urinary tract and upper respiratory tract
injury, poisoning, and procedural complications	radiation recall dermatitis
investigations	***ECG abnormalities*** (8-14%, severe <1%) , see paragraph following **Side Effects** table
	alkaline phosphatase, elevated (18-22%, severe 1%)
	AST, elevated (18-19%, severe 1%)
	bilirubin, elevated (4-7%, severe 1%)
musculoskeletal and connective tissue	***arthralgia/myalgia*** (54-60%, severe 8-12%) ; see paragraph following **Side Effects** table
nervous system	autonomic neuropathy, resulting in paralytic ileus and orthostatic hypotension (<1%)
	motor neuropathy, with resultant minor distal weakness (<1%)
	peripheral neuropathy (52-64% severe 2-4%) ; see paragraph following **Side Effects** table
respiratory, thoracic and mediastinal	dyspnea (2%)
	radiation recall pneumonitis
skin and subcutaneous	***alopecia*** (87-93%) ; usually complete, generally occurs 14-21 days after
	nail discolouration (2%)
	rash (12-14%)
vascular	hypotension (11-24%); during first 3 h of infusion
	phlebitis

3.2. Drug Interactions

Paclitaxel, belonging to the class of taxanes, has demonstrated extraordinary activities in clinical trials against a variety of tumors, including gastric cancer, colon cancer, breast cancer etc. Paclitaxel is a mitotic spindle poison that accelerate the microtubule assembly from tubulin and block the depolymerization of the microtubule.

Paclitaxel is presently formulated as Taxol, a concentrated solution containing 6 mg of paclitaxel/ml of Cremophor EL. (Polyoxyethylated castor oil and ethanol, 50% v/v) that must be further diluted before administration.

The major drawbacks associated with paclitaxel are, severe side effects such as, toxic effects on bone marrow, neutropenia, hypersensitivity reactions, limited water solubility etc.

Hence in present work an attempt is being made to provide an alternative drug delivery system having improved therapeutic index for paclitaxel in the form of novel polymeric thermosensitive micellar delivery system which have following advantages,

- Improved site specificity.
- Localization of the drug in the tumor cells by thermal targeting.
- Reduce risk of unwanted side effects.
- Increase bioavailability and hence smaller dosage form size.
- Possibility of higher dosage without side effects.
- More effective cancer treatment.

Table 4: Drug Interactions of Paclitaxel

INTERACTIONS:

AGENT	EFFECT	MECHANISM	MANAGEMENT
cisplatin	may increase neutropenia when paclitaxel is given *after* cisplatin	paclitaxel clearance is decreased by 25-33% when given *after* cisplatin	preferred method is to give paclitaxel first when administering as sequential infusions
dexamethasone	does not affect protein binding of paclitaxel		
diphenhydramine	does not affect protein binding of paclitaxel		
disulfiram	development of acute alcohol intolerance reactions	inhibition of aldehyde dehydrogenase by disulfiram, leading to development of toxic metabolites of ethanol (found in the solution)	avoid disulfiram concurrently with paclitaxel administration
doxorubicin	may increase cardiac toxicity from doxorubicin when given concurrently with paclitaxel	doxorubicin clearance is decreased leading to increased plasma levels of doxorubicin and doxorubicinol	monitor for increased cardiotoxicity
metronidazole and derivatives	development of acute alcohol intolerance reactions; the risk for most patients appears slight	inhibition of aldehyde dehydrogenase by metronidazole, leading to development of toxic metabolites of ethanol (found in solution)	avoid metronidazole and its derivatives concurrently with paclitaxel administration
vaccines, live	enhanced viral replication may increase the risk of disseminated disease	decreased immune response allows live vaccine to produce infection	avoid live vaccines during treatment
warfarin	may increase anticoagulant effect of warfarin when given concurrently with paclitaxel	paclitaxel may displace warfarin from plasma protein binding sites when given concurrently	monitor INR and adjust warfarin dosing accordingly; consider use of LMWH with chemotherapy

3.3 Precautions

Caution

Preexisting liver impairment may impair elimination of paclitaxe reduction is suggested

Special populations

Elderly patients may have more myelosuppression, neuropathy and cardiovascular toxicities

Patients with AIDS-related Kaposi's sarcoma may have more hematologic toxicities, infections and febrile neutropenia.

Carcinogenicity

No information found

Mutagenicity

Not mutagenic in Ames test and mammalian *in vitro* mutation test. Paclitaxel is clastogenic in human lymphocytes *in vitro* but not in other mammalian *in vivo* chromosome tests.

Fertility

In animal studies, reduced fertility has been observed, with decreased pregnancy rates and increased embryo loss in females and testicular atrophy / degeneration in males.

Pregnancy

FDA Pregnancy Category D.5,10 There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g., if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective). Paclitaxel has shown to be embryotoxic and fetotoxic in animal studies; soft tissue and skeletal malformations have been reported. Breastfeeding is not recommended due to the potential secretion into breast milk.

3.4. Side effects

- Low blood counts. Your white and red blood cells and platelets may temporarily decrease. This can put you at increased risk for infection, anemia and/or bleeding.
- Hair loss

- Arthralgias and myalgias, pain in the joints and muscles. Usually temporary occurring 2 to 3 days after Taxol, and resolve within a few days.
- Peripheral neuropathy (numbness and tingling of the hands and feet)
- Nausea and vomiting (usually mild)
- Diarrhea
- Mouth sores
- Hypersensitivity reaction - fever, facial flushing, chills, shortness of breath, or hives after Taxol is given. The majority of these reactions occur within the first 10 minutes of an infusion. Notify your healthcare provider immediately (premedication regimen has significantly decreased the incidence of this reaction).

The following are less common side effects (occurring in 10-29%) for patients receiving Taxol:

- Swelling of the feet or ankles (edema).
- Increases in blood tests measuring liver function. These return to normal once treatment is discontinued. (see liver problems).
- Low blood pressure (occurring during the first 3 hours of infusion).
- Darkening of the skin where previous radiation treatment has been given (radiation recall - see skin reactions).
- Nail changes (discoloration of nail beds - rare) (see skin reactions).

3.5 Uses

Primary use:

- Breast cancer
- Lung cancer-non small cell.
- Ovarian cancer,
- Kaposis sarcoma.

Other uses:

- Lung cancer.
- Esophageal cancer.
- Bladder cancer.
- Head and neck cancer.
- Cervical cancer.
- Endometrial cancer.

Aim of the present investigation

Drug delivery systems to the colon are being actively investigated in order to develop oral preparations of peptides and treat local colonic diseases, e.g., irritable bowel syndrome, ulcerative colitis, cancer, and infection.

Paclitaxel is a microtubule-stabilizing agent which promotes polymerization of tubululin causing cell death by disrupting the dynamics necessary for cell division. It has neoplastic activity especially against primary epithelial ovarian carcinoma, breast, colon, and non-small cell lung cancers. Paclitaxel is poorly soluble in aqueous solutions but soluble in many organic solvents such as alcohols. It therefore lends itself well to more advanced formulation strategies. In the present investigation, an attempt was made to prepare Paclitaxel Silver Nanoparticle by precipitation technique. In order to stabilize the size of particle, various concentrations of Silver nitrate and Trisodium citrate to be used. Prepared Nanoparticle to be optimized by their encapsulation efficiency, particle size and Release rate. Zeta potential and SEM study also included in the investigation. A short term stability study proposed for optimized formulation.

Plan of Work

1. **Literature survey**

   a) Selection of drug

   b) Selection of polymers

   c) Selection of suitable method

2. **Procurement of Drug, Polymer and excipients**.
3. **Raw material Analysis**.
4. **Preformulation studies**

   a) Solubilitystudy.

   b) Comparatibility studies- The drug and polymer compatibilitycan be done with the FTIR.

5. **Construction of Calibration Curve**.
6. **Preparation of paclitaxel Nanoparticle by Precipitation Technique.**
7. ***In vitro* drug release study for prepared Nanoparticles.**
8. **Determination of Particle Size Analysis and Encapsulation Efficiency**.
9. **Analysis for Zeta Potential.**
10. **SEM Analysis.**
11. **Stability Study.**

Materials and Methods

Materials used in the formulation of Paclitaxel silver Nitarte Nanoparticles.

Table 5

Materials	Manufacturers/Suppliers
Paclitaxel	Salius Pharma Pvt.Ltd (Mahapi Navi Mumbai)
Silver Nitrate	Merck Life Science Private Limited (Mumbai)
Tripoly Citrate (TPP)	Thermo Fisher Scientific India Pvt. Ltd (Mumbai)
Methanol	Changshu Hongsheng Fine Chemical Co.Ltd .(China)
Acetone	S.D.Fine chemicals Ltd (Mumbai)
Dichloromthane	S.D.Fine chemicals Ltd (Mumbai)
Isopropyl alcohol	Chemspure (Chennai)
N-hexane	S.D.Fine chemicals Ltd (Mumbai)
Buty 4- Aminobenzoate(Butamben)	S.D.Fine chemicals Ltd (Mumbai)
Starch	S.D.Fine chemicals Ltd (Mumbai)
Hydroxy propyl Methyl cellulose	Chemspure (chennai)

Equipments used in the formulation of Paclitaxel silver Nitrate Nanoparticles

Table 6

Instruments	Manufacturer
Digital balance	Axis,India
Dissolution testing apparatus	Electrolab India Pvt Ltd
UV-Visible spectrophotometer	Shimadzu Analytaical (India) Pvt Ltd
Fourier Transform Infrared Spectroscopy	ABB Bomem ,Quebee,Canada
Scanning Electron Microscop (SEM)	FEI,Quanta 200 SEM,USA
Heating Mantle	Sigma instruments (Chennai)
Sigma Humidity Chamber(40C 75%/RH)	Sigma scientific product ,Chennai
High Performance Liquid Chromatography(HPLC)	SHIMADZU SPD-20 A(PDA Dector),India
Particle size analyzer	(Beckman Coulter Delsa Nano C, Brea, USA).
Zeta potentiometer	(Beckman Coulter Delsa Nano C, Brea, USA).

6.1 Raw material analysis of Paclitaxel:[48]

6.1.1 Description:

White colour powder ;odourless.

6.1.2 Melting point:

Melring point of Paclitaxel was determined by capillary method.

6.1.3 Precentage purity:[48]

Assay:

Procedure—separately an equal volumes were injected (about 10 µL) of theStandard preparationand theassay preparationinto the chromatograph, the chromatograms was recorded, and measured the areas for the major peaks. The quantity was calculated, in mg, of $C_{47}H_{51}NO_{14}$ in the portion of Paclitaxel taken by the formula: In which C is the concentration, in mg per ml, of USP Paclitaxel RS in the Standard preparation;and ru and rs are the peak responses for paclitaxel obatined from the assay preparation and the satandard preparation,respectively.

6.2 Identification test for paclitaxel

Table 7 : Identification test of paclitaxel

S.No	Test	Method
1.	**Infrared Absorption**	As per USP
2.	**Retention Time**	As per USP

6.3 Organoleptic Characteristics

The color, odor, and taste of the drug were characterized and recorded using descriptive terminology.

6.4 Preformulation Studies:

Preformulation is defined as the stage of research and development process where physical, chemical and mechanical properties of a new drug substance are characterized alone and when combined with excipients in order to develop stable, safe and effective dosage form.[51]

A thorough understanding of physicochemical properties may eventually provide a rationale for formulation design or carry, the need for molecular modification or purely confirms that there are no significant barriers to the compound development. Hence, preformulation studies were performed on the obtained sample of drug for solubility analysis, identification and compatibility studies.

6.4.1 Solubility analysis

The solubility of Paclitaxel was performed by using traditional shaker agitator.100 mg of Paclitaxel was added to 5 ml of methanol and was shaken on a mechanical shaker for 48 hours at 37^0C. After 48 hours , the resultant saturated dispersion in filtered using 0.45 µfilter followed by 0.2 µ filter to exclude the undissolved solids.The filtrate obtained was diluted appropriately using suitable solvent and estimated for drug content using UV spectrophotometer/HPLC.

6.4.2 FTIR Spectral analysis[50]

FTIR spectral analysis of pure drug and polymers was carried out and observation was made whether changes in the chemical constitution of drug after combining it with the polymers occurred. About 2 mg of the samples were mixed with potassium bromide of equal weight and crushed to get pellets applying pressure on 600 Kg/cm^2

and scanned with the IR instrument (Shimadzu, 8400 Series,Tokyo, Japan) from 400-4000cm^{-1}.

6.4.3 Calibration curve

The calibration curve for paclitaxel was prepared by using phosphate buffer (pH 7.4).**Phosphate buffer pH 7.4**

Phosphate buffer was prepared by placing 25ml of 0.2M potassium dihydrogen orthophosphate solution and 19.55ml of 0.2N sodium hydroxide solution in a 100ml volumetric flask and volume was made up to the mark with distilled water. The pH was found to be 7.4±0.1.

Primary stock solution for paclitaxel with phosphate buffer (pH 7.4)

Accurately weighed 100mg of paclitaxel was dissolved in 10ml of methanol in a 100ml of volumetric flask and volume was made up to the mark with pH 7.4 buffer to get a concentration of 1000µg/ml.

Secondary stock solution for phosphate buffer (pH 7.4)

From primary stock solutions of phosphate buffer 10ml was pipetted out in a 100ml of volumetric flask and volume was made up to the mark with pH 7.4 buffer to obtain a concentration of 100µg/ml.

From secondary stock solutions, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0ml were piped out and diluted to 10ml with respect buffers to get a concentration range from 5µg/ml to 50µg/ml. Then it was analyzed spectrometrically at 220nm.

The calibration curve was plotted with the absorbance Vs concentration (µg/ml).

6.5 Method of preparation for Paclitaxel silver nitrate Nanoparticles by precipitation Technique:

Silver nitrate and trisodium citrate were used as starting materials for the preparation of Paclitaxel silver nitrate Nanoparticles (NP). The silver colloid was prepared by using chemical Precipitation method. All solutions of reacting materials were prepared in distilled water. In typical experiment 50 ml of 0.001 M $AgNO_3$ was heated to boil. To this solution 5 mL of Paclitaxel (150 mg/5 ml of methanol) solution was added followed by addition of 5 ml of 1 % of trisodium citrate added drop by drop. During the process, solutions were mixed vigorously and heated until change of color was evident (pale yellow). Then it was removed from the heating device and stirred until cooled to room temperature. The colloidal solution of silver Nanoparticles were characterized by using UV-Visible spectroscopy and SEM. The entire addition process took about 3 minutes, after which the stirring was stopped and the stir bar was removed. Reaction conditions including stirring time and relative quantities of reagents (both the absolute number of moles of each reactant as well as their relative molarities) must be carefully controlled to obtain stable yellow colloidal silver. If stirring was continued once all of the silver nitrate was added, aggregation began as the yellow solution first turned to darker yellow then violet and eventually grayish after which the colloid broke down and particle settled out.

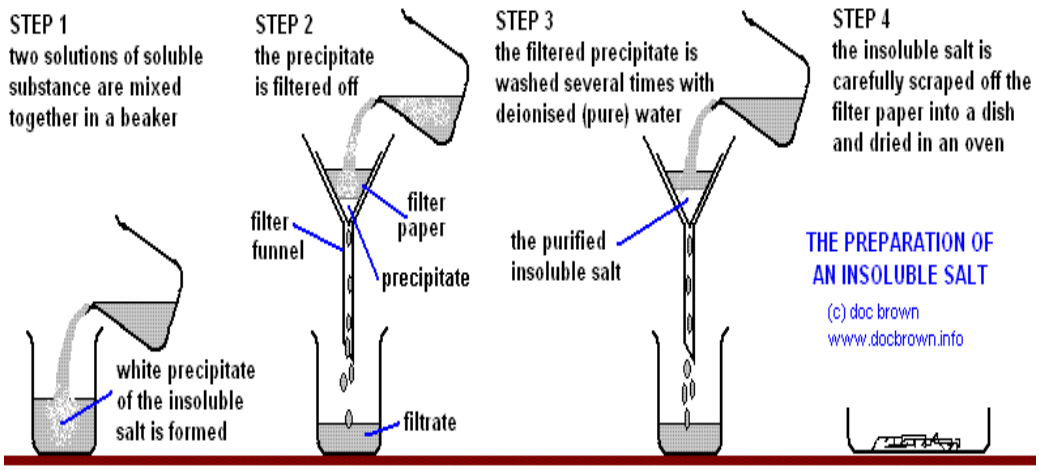


Fig.7: Diagrammatic Representation of Method of Preparation of paclitaxelNanoparticles By preciptation Method.

Fig.8: Photograph showing the preparation of Nanoparticles

Fig.9: Silver Nitarte Nanoparticles Product

Table 8: List of excipients and their use

S.No	Name	Use
1	**Paclitaxel**	Anti-Cancer
2	**Silver nitrate**	Natural Polymer
5	**Dichloromethane**	Organic solvent
6	**Trisodium citrate**	Crosslinking agent
8	**Methanol**	Organic solvent

Table 9: Formulationtablefor Paclitaxel Nanoparticles

Formulation Code	Drug (mg)	$AgNO_3$ (mg)	Molarity	Methanol	Trisodium citrate (1% w/w)
PXN1	150	0.017gm	0.0001M	5	5
PXN2	150	0.17gm	0.001M	5	5
PXN3	150	0.5gm	0.003M	5	5
PXN4	150	0.8 gm	0.004M	5	5
PXN5	150	1.0 gm	0.005M	5	5
PXN6	150	1.17gm	0.00688M	5	5
PXN7	150	1.17gm	0.00688M	5	10
PXN8	150	1.17gm	0.00688M	5	3

6.6 Evaluation of Paclitaxel silver nitrate Nanoparticles:

6.6.1 Apparent bulk density:

The bulk density was determined by transferring the accurately weighed sample of powder to the grounded cylinder. The initial volume and weight was noted. Ratio of weight of sample was calculated by using the formula

Density= Mass/Volume

6.6.2 Tapped density:

Weighed powder sample was transferred to graduated cylinder and was placed on the tap density apparatus, was operated for fixed number of taps (500 times).The density was determined by the formula.

Density=Mass/Tapped Volume

6.6.3 *Carr's Index:*

Based on the poured density and tapped density, the percentage compressibility of the granules was computed using the carr's index by the formula and the carr's index value and its specifications are given in **Table10**

$$\text{Carr's index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \times 100$$

Table 10: Carr's index value

S No	%compressibility	Type of flow
1	5-15	Excellent
2	12-16	Good
3	18-21	Fair
4	23-25	Poor
5	33-38	Very poor
6	>40	Extremely poor

6.6.4 Haunser' s ratio

It indicates the flow properties of the powder and is measured by the ratio of tapped density to bulk density **Table 11**

$$\textbf{Hausner's ratio} = \frac{\textbf{Tapped density}}{\textbf{Bulk density}}$$

Table 11 : Haunser's ratio

Haunser's ratio	**Type of flow**
<1.25	Good flow
>**1.25**	Poor flow

6.6.5 ***Porosity:***

Paclitaxel powder is placed in a graduated cylinder and the total volume is noted. The volume occupied is known as the bulk volume, V_b. If the powder is nonporous, that is ,has no internal pores or capillary spaces, the bulk volume of the powder consists of the true volume of the solid particles plus the volume of the spaces between the particles. The volume of the spaces, known as the void volume v,is given by the equation

$$\mathbf{V = V_b \_ Vp}$$

Where V_p is the true volume of the particles. The method for determining the volume of the particles will by given later.

The porosity or voids. Of the powder is defined as the ratio of the void volume to the bulk volume of the packing:

$$\varepsilon = \frac{v_b - v_p}{v_b} = \frac{1 - v_p}{v_b}$$

6.6.6 Drug content and encapsulation efficiency[55]

Precisely weighed 100mg of Paclitaxel Silver Nitrate Nanoparticles were crushed in a mortar and suspended in 100ml of phosphate buffer (pH 7.4) and kept in sonication for 2hrs. Then the samples were centrifuged at 1000rpm for 20mins to remove the supernatant layer, if any. The samples were filtered. From this filtered solution 1 ml of sample was withdrawn and diluted to 100 ml with phosphate buffer (pH 7.4). Then it was analyzed spectrophotometrically at 271nm.

6.4.10 Drug content:

Theoretical drug content =Weight of drug loaded / Total weight of Nanoparticles

Practical drug content = Concentration X dilution factor X Conversion factor

Encapsulation efficiency = (Actual drug content / Theoretical drug content) X 100

6.7 In-vitro Drug Release Studies[56]

In vitro release of Paclitaxel Silver Nitrate Nanoparticles was conducted by a dialysis membrane having a pore size of 2.4 mm (LA-395-5Mt Himedia Pvt. Ltd, Mumbai, India) with 75 ml of pH 7.4 phosphate buffer at 37°C. Briefly in a 100 ml beaker 75ml of pH 7.4 phosphate buffer was taken. A 2 ml of formulation was taken into a dialysis bag and dipped into the buffer solution. The dialysis membrane was activated earlier using by soaking in 1% w/v NaOH overnight. The flask was kept on a magnetic stirrer. Stirring was maintained at 250 rpm and the temperature of the buffer was maintained at 37°C. Sampling was done by withdrawing 1 ml of aliquots from a beaker. Immediately 1 ml of new buffer was added to keep the sink condition. Samples were analyzed after sufficiently diluting with methanol by using a UV/Spectrophotometer (UV/VIS-Double beam Spectrophotometer, V-530, Jasco, Tokyo, Japan) at a wavelength of 271 nm. Each test was conducted thrice and average value taken for the calculation.

Fig.10: Dialysis Membrane

6.*7.1 Amount of drug present*

= Concentration × Dilution factor × Conversion factor × Amount of stock solution.

$$\text{Cumulative \% drug release} = \frac{\text{Amount of drug present}}{\text{Amount of drug to be present}} \times 100$$

The release data obtained were fixed into various mathematical models like zero order, First Order, Higuchi and Korsmeyer-Peppas to know which mathematical model was best fitting the obtained release profile.

6.7.2 Kinetic study: Zero order release

In order to analyze the release mechanism, several release models were experiencing such as

$$\textbf{Zero order } Q_t = Q_0 + K_0 t$$

Where, Q_t is the amount of drug released at time t,

K_0 is the apparent dissolution rate constant or zero order release constant and

Q_0 is the initial concentration of the drug in the solution resulting from a burst effect; in this case the drug release runs at a constant rate.

It describes the systems where the drug release rate is autonomous of its concentration of the dissolved substance.

A graph is plotted between the time taken on x-axis and the cumulative % of drug release on y-axis and it gives a straight line.

6.7.3 Application in first order release:

This relationship can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms, osmotic systems, etc .[57, 58]

The release of the drug which followed first order kinetics can be expressed by the equation:

$$dc/dt = -Kc \quad (1)$$

Where K is first order rate constant expressed in units of $time^{-1}$.

Equation (1) can be expressed as:

$$\log C = \log C_0 - Kt / 2.303 \quad (2)$$

Where C0 is the initial concentration of drug, k is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time, which would yield a straight line with a slope of -K/2.303.

6.7.4 Application in Higuchi release equation:

This relationship can be used to explain the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in permeable matrices.[59, 60]

The Higuchi release equation is

$$\mathbf{Q = K_H t^{1/2}}$$

Where

Q is the cumulative amount of drug release at time "t"

K_H is Higuchi constant

T is time in hours

The Higuchi equation suggests that drug release by diffusion.

A graph is plotted between the square root of time taken on x-axis and the cumulative percentage of drug release on y-axis and it gives a straight line.

6.7.5 Application in korsmeyer-peppa's equation :

This relationship can be used to illustrate the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs**.**[61, 62]

The korsmeyer-peppas equation was given as

$$\mathbf{F = (M_t/M) = K_m t^n}$$

Where

F is fraction of drug released at time't'

Mt is amount of drug release at time't'

M is total amount of drug in dosage form

K_m is kinetic constant

n is diffusion or release exponent

t is time in hours

n is estimated from linear regression of log (Mt/M) versus log t

If n = 0.45 it indicates fickian diffusion

0.45<n<0.89 it indicates anomalous diffusion or non-fickian diffusion

Anomalous diffusion or non-fiction diffusion refers to a combination of both diffusion and erosion controlled rate rules.

A graph was plotted between the log time release on x-axis and the log cumulative percentage of drug release on y-axis and it gives a straight line.

Interpretation of drug release mechanism is given in following **Table 12.**

Table 12: Interpretation of Drug Release Mechanism

Release exponent (n)	Drug release mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0..5}$
0.5 < n <1	Anomalous transport	t^{n-1}
1	Case-II transport	Zero order release
> 1	Super case II transport	t^{n-1}

.6.7.6 SEM Analysis[52]

Scanning electron microscopy (JEOL 5400, Tokyo, Japan) was used to decide the shape, surface topography and texture as well as to inspect the morphology of cracked or sectioned surface. SEM is a frequently used method for characterizing drug delivery systems, owing in large part to simplicity of sample preparation and ease of operation. Sample spreads on the small square plate and coated with a gold ion for 5-6 mins. The prepared sample was kept inside the chamber and images captured with different magnifications.(10,000, 15,000 and 20,000)

6.7.7Particle Size Analysis (PSA)[53]

The size division of the Nanoparticles was determined using the particle size analyzer (Beckman Coulter, Delsa nano C, Brea, USA) prepared with a dry accessory system. About 2ml of the prior prepared suspension has to be transferred into a 4.5 ml disposable plastic cuvette, placed in the analysis device and subsequent analyzed for size analysis and temperature maintained at 25°C.

6.7.8 Zeta Potential Analysis[54]

The zeta potential was measured using the appropriate instrument (Beckman Coulter Delsa Nano C, Brea, USA).using automatic titration regime that adjusts the pH of the sample to pre-defined values by adding 0.1M HCL or 0.1M NaOH titrator a volume of 20 ml suspension is necessary.

Zeta Potential (mV)	**Stability behavior of colloid**
From 0 to ±5	Rapid coagulation or Flocculation
From ±10 to ±30	Incipient instability
From ±30 to ±40	Moderate stability
From ±40 to ±60	Good stability
More than ±61	Excellent stability

6.7.9 Stability studies :

The objective of the stability testing is to show evidence as how the quality of the drug substance or product varies with time under the influence of variety of environmental factors such as temperature, humidity and light to establish a reset for the drug substance or a shelf life for the drug product and recommended storage condition.

Stability of a drug is defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutically and toxilogical specifications.

The optimised batch paclitaxel silver nitare Nanoparticles were subjected for accelerated stability studies. The Paclitaxel Nanocapsule from the batch of PXN6filled in the capsule (size1) equivalent to 150 mg. Lactose was used as diluent to make up total weight of capsule as 290 mg (161+129).The Paclitaxel Nanocapsules were kept in in sigma stability chamber. The samples were analysed at 0,1 and 2 months intervals. The data was analysed for any significant changes from the initial data. The following test were performed:

1. Test for physical parameters
2. Assay
3. *In-vitro* dissolution study.

Results

7.1 Raw material analysis of Paclitaxel:[51]

7.1.1 *Description:*

White colour powder ;odourless.

7.1.3 *Precentage purity:*

Assay: **Identification test for paclitaxel**

Table 13: Identification test of paclitaxel

S No	Test	Method
1.	**Infrared Absorption**	As per USP
2.	**Retention Time**	As per USP

7.2 Preformulation Studies:

7.2.1 Organoleptic Characteristics

The color, odor, and taste of the drug were characterized and recorded using descriptive terminology; the results were shown in the **Table 14**.

Table 14: Organoleptic Charecteristics of Paclitaxel

Properties	Result
Odour	Odourless
Colour	White to off-white
Form	Crystalline

7.2.2 Melting point

Table 15: Melting point of the pure paclitaxel

Sample	Reported	Observed
Paclitaxel	216-217 °C	^{0}C

7.2.3 Solubility :

Table 16:Solubility of Paclitaxel in different solvents.

S.No	Solvent	Pure Drug	Solubility
1.	**Methanol**	(50 mg/ml)	Freely soluble
2.	**Dichloromethane**	(5mg/ml)	Soluble
3.	**Ethanol**	(1.5mg/ml)	Soluble
4.	**Octanol**	(0.4mg/ml)	Slightly soluble
5.	**Ethyl acetate**	(0.25mg/ml)	Slightly soluble
6.	**Water**	(10-20 µM)	Poorly soluble

7.4 FT-IR spectral analysis

The FT-IR analysis of the drug and polymer gave thermal profile charecterstic of the substances.

Fig.11: FTIR Spectrum of Pure Drug paclitaxel

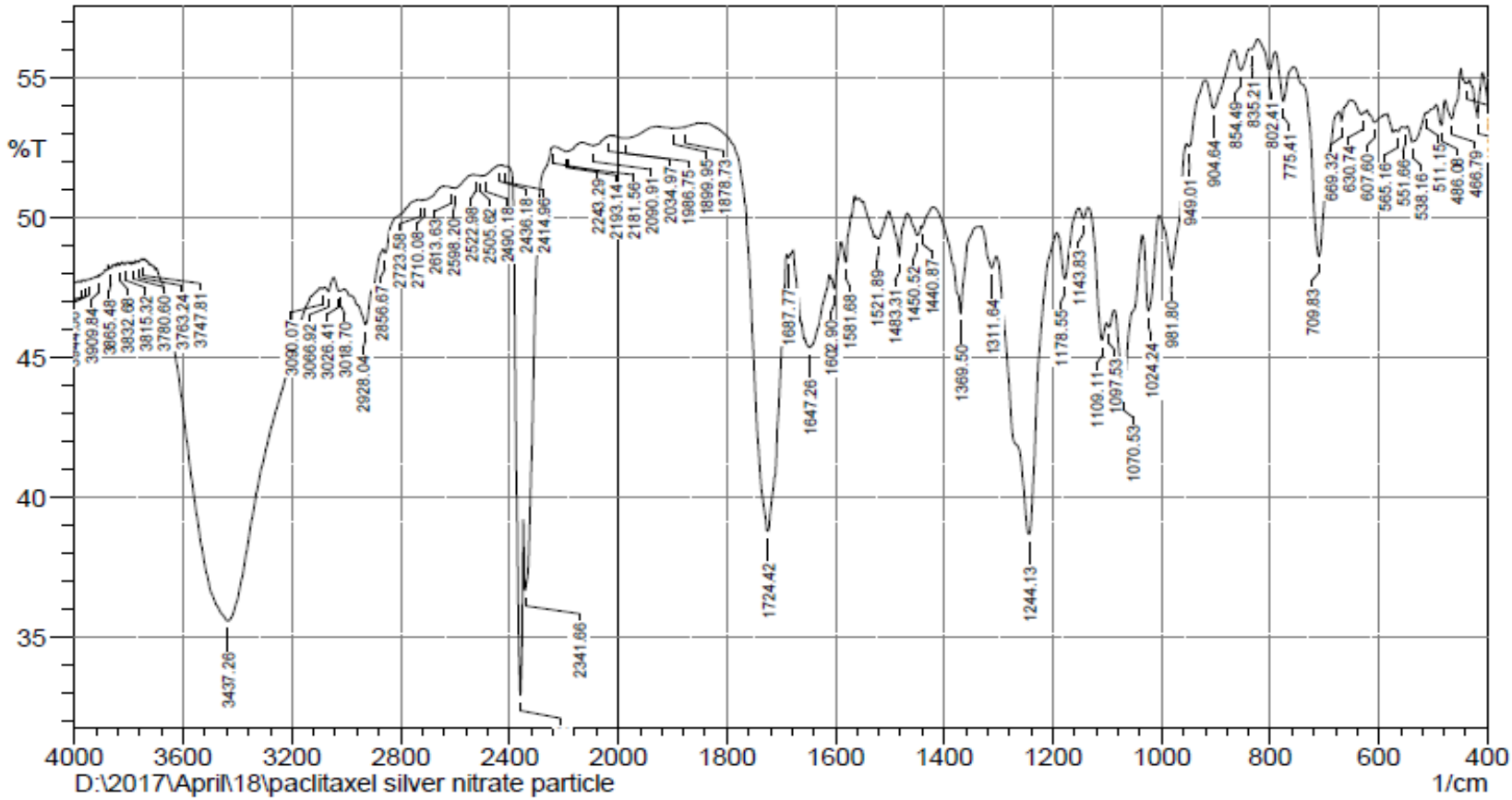


Fig.12: FTIR Spectrum of Formulation paclitaxel

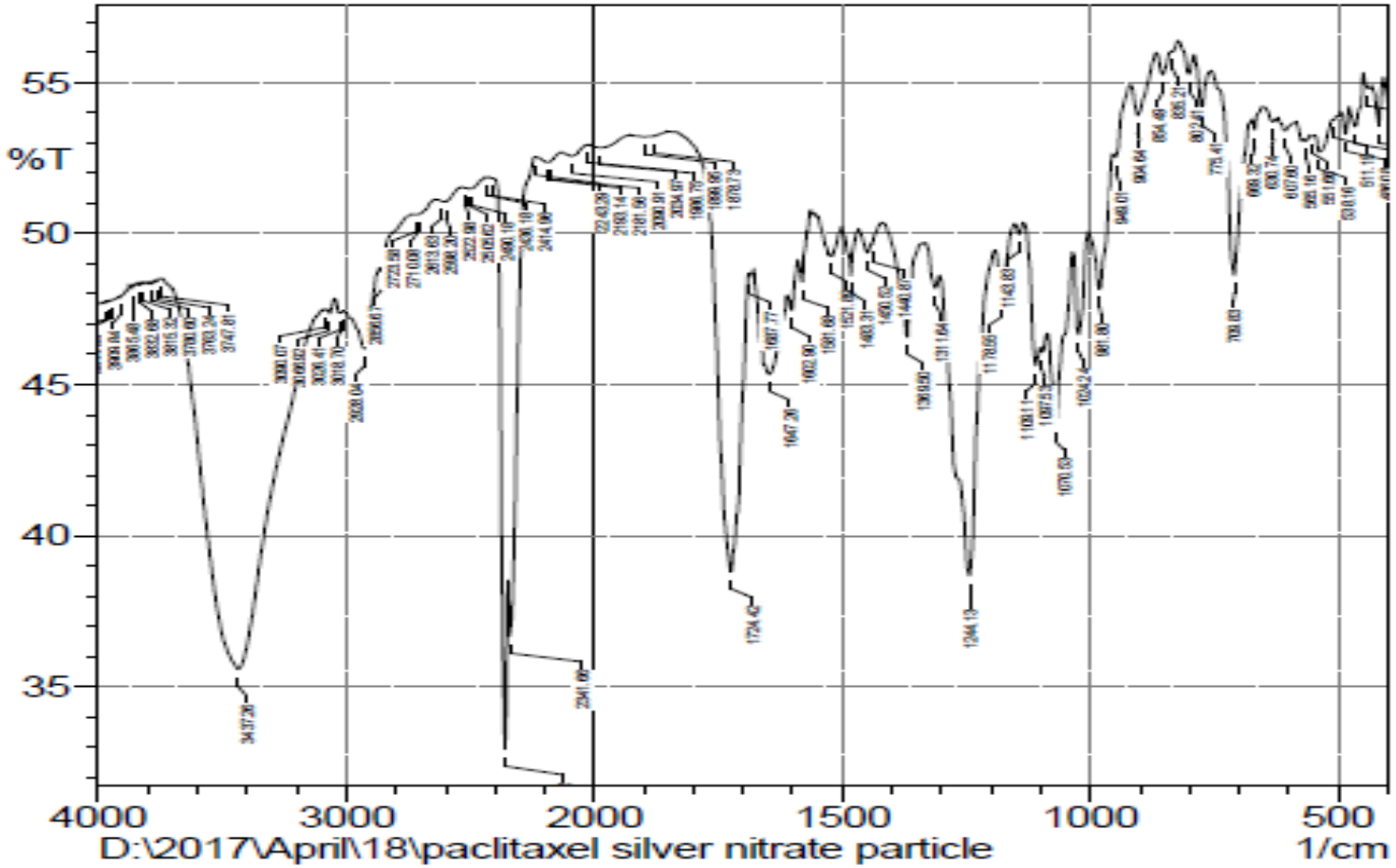


Table 17 : Comparison of FTIR peaks of drug and polymers

S.No	Formulation	Wave number (cm^{-1})
1	**Pure Drug paclitaxel**	3437.26, 2928.04, 2341.66, 1724.42, 1647.26, 1369.50, 1244.13, 1070.53, 709.83
2	**Paclitaxel silver nitrate particle**	3437.26, 2341.66, 1724.42, 1244.13, 1070.53, 709.83

8.3 Standard calibration curve of paclitaxel in (pH 7.4) buffer solutions at 265**nm:**

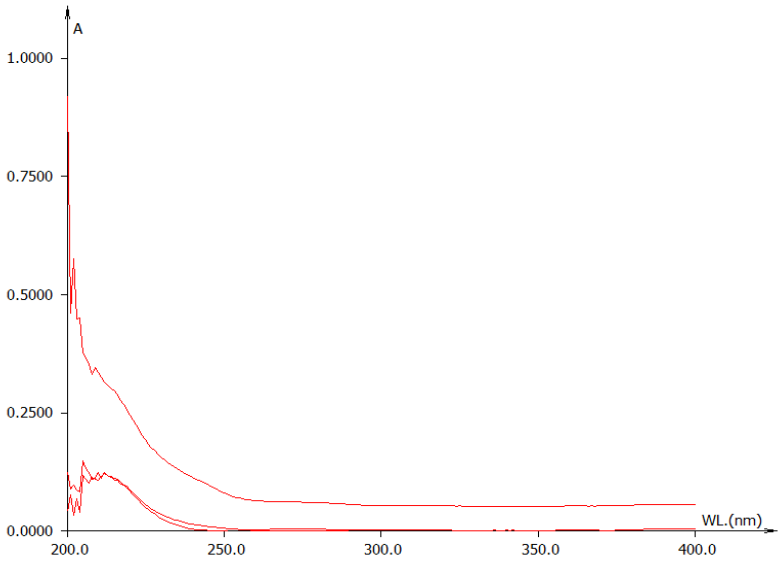


Fig.13: λmax of in pH 7.4 at 220nm

Table 18: Obervations for Standard graph of in pH 7.4 at 220nm

Sl.No	Concentration (µg/ml)	Absorbance
1	**0**	0
2	**0.3**	0.156
3	**0.6**	0.295
4	**0.9**	0.451
5	**1.2**	0.591

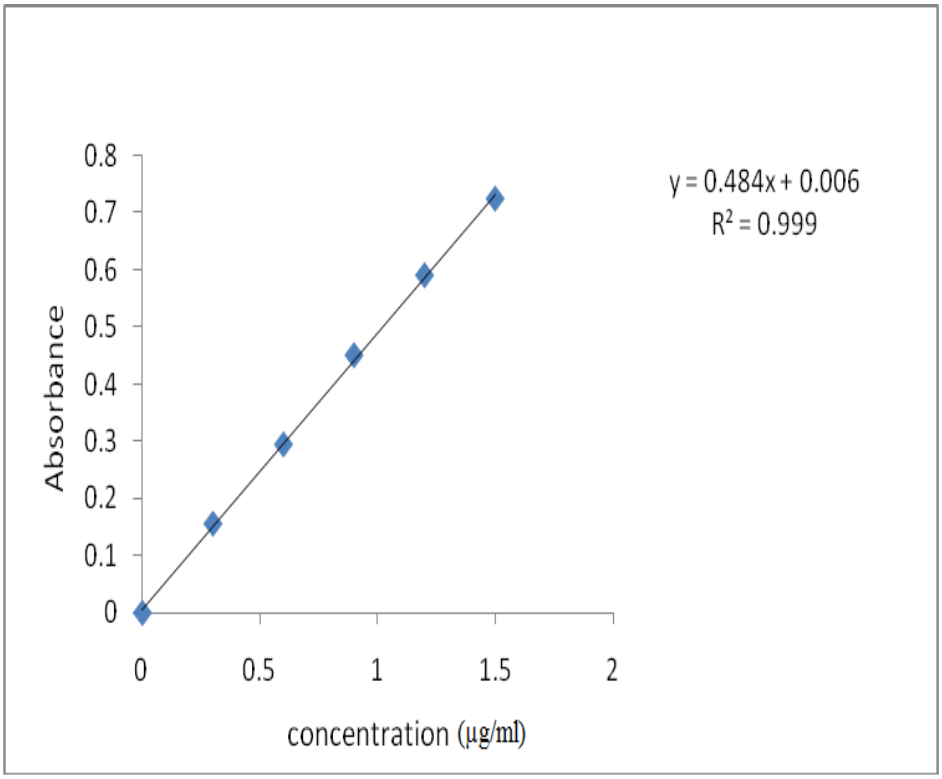


Fig.14: Calibration curve of paclitaxel in pH 7.4 at 220nm

7.4 Optimization:

Table 19 : Micrometric properties of optimized paclitaxel Nanoparticles

S No	Parameters	Report
1	**Tapped Density**	0.563±0.04 (gm/ml)
2	**Bulk Density**	0.584±0.01 (gm/ml)
3	**Carr's index**	3.69±0.4 %
4	**Haunser's ratio**	1.038±0.02

Table 20: *Invitro* Release Profile of Paclitaxel Nanoparticle from Formulation PXN-1

***In vitro* drug release data**		**Higuchi's data**		**Peppa's data**	
Time (hrs)	**Cumulative % drug release**	**Square root time**	**Cumulative % drug release**	**Log time**	**Log cumulative % drug release**
1	12.09	1	12.09	0	1.08
2	20.73	1.41	20.73	0.30	1.31
3	29.87	1.73	29.87	0.47	1.47
4	38.04	2	38.04	0.60	1.58
5	45.22	2.23	45.22	0.69	1.65
6	50.53	2.44	50.53	0.77	1.70
7	56.00	2.64	56	0.84	1.74
8	59.36	2.82	59.36	0.90	1.77
9	64.90	3	64.90	0.95	1.81
10	69.24	3.16	69.24	1	1.84
12	74.64	3.46	74.64	1.07	1.87
24	98.50	4.89	98.50	1.38	1.99

n = 3

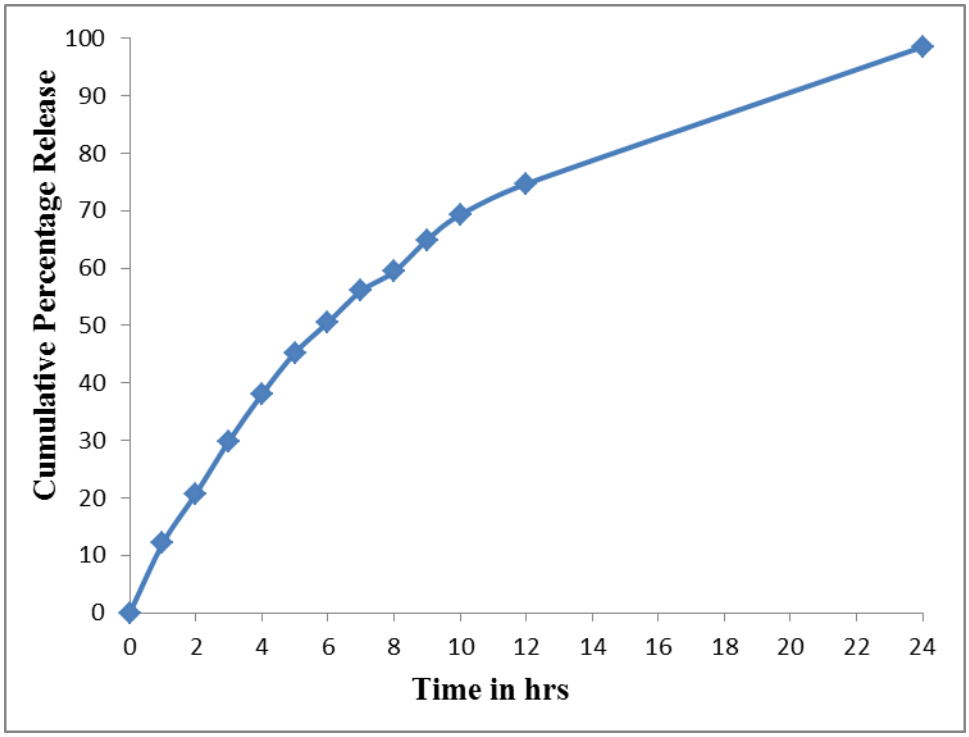


Fig.15: Zero Order Release Plot for Formulation PXN-1

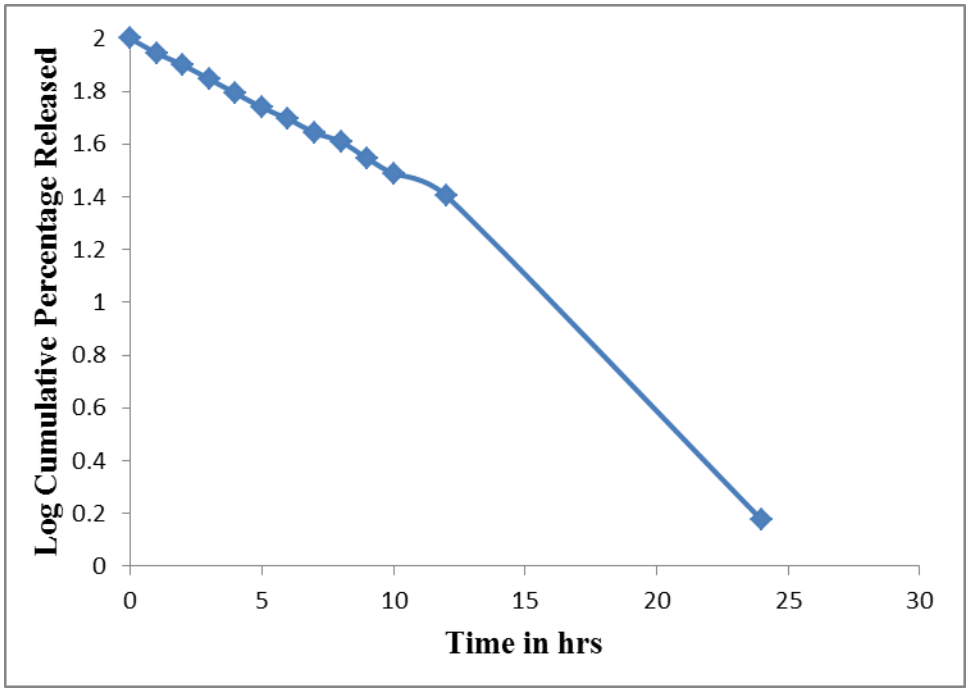


Fig.16: First order Release Plot for Formulation PXN-1

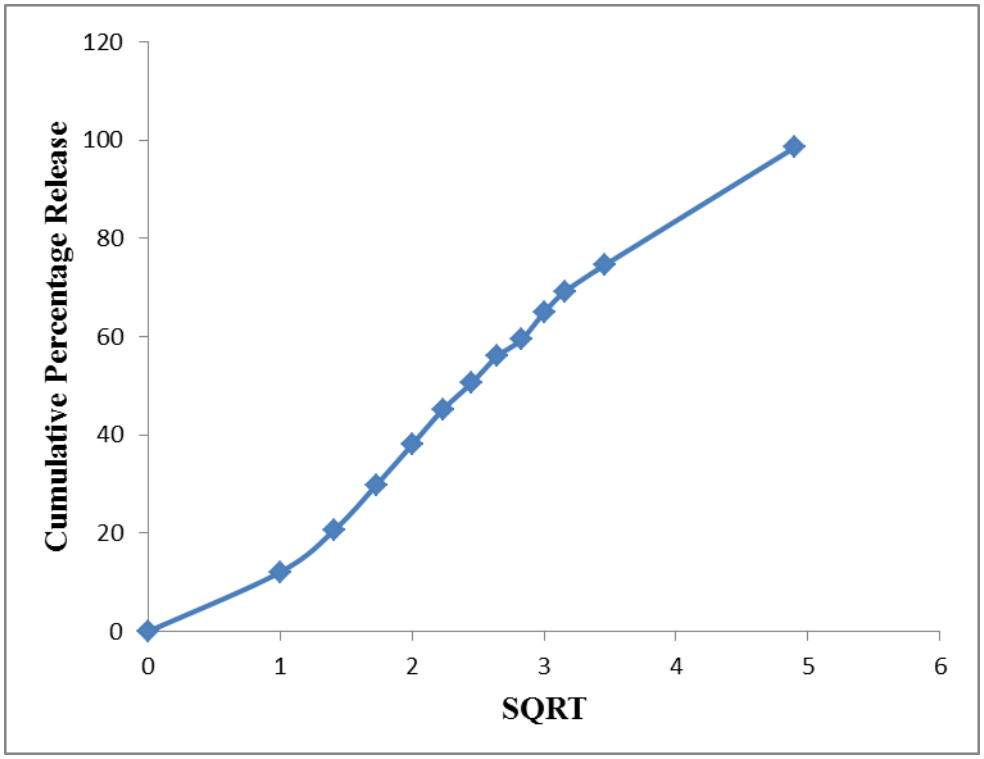


Fig.17: Higuchi Release Plot for Formulation PXN-1

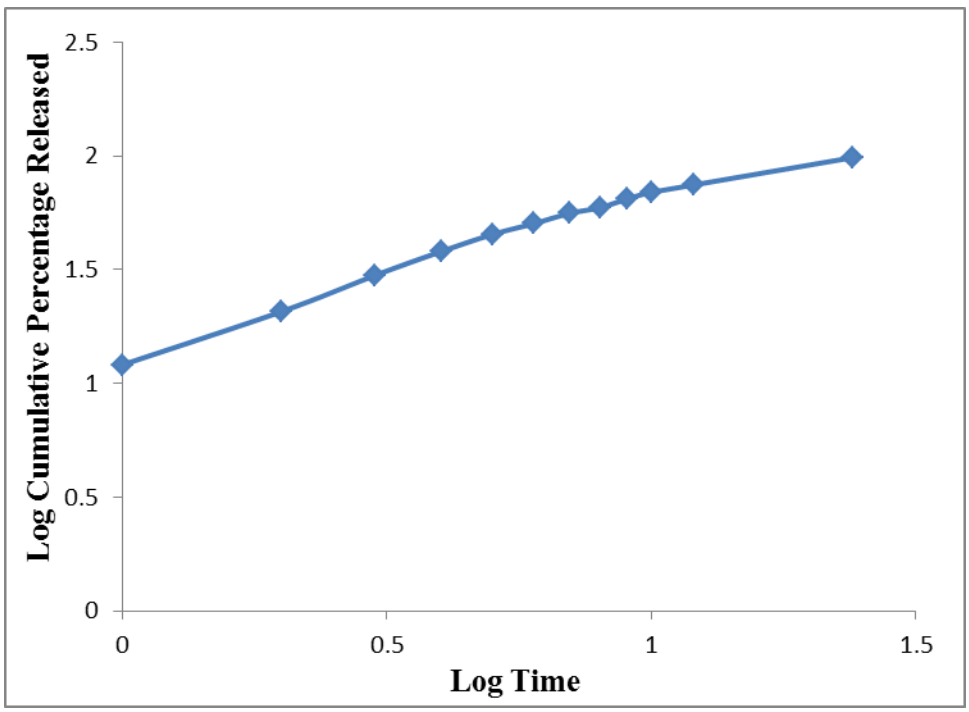


Fig.18: Peppas Release Plot for Formulation PXN-1

Table 21: *Invitro* Release Profile of Paclitaxel Nanoparticle from Formulation PXN-2

***In vitro* drug release data**		**Higuchi's data**		**Peppa's data**	
Time (hrs)	**Cumulative % drug release**	**Square root time**	**Cumulative % drug release**	**Log time**	**Log cumulative % drug release**
1	**9.69**	**1**	**9.69**	**0**	**0.98**
2	**19.47**	**1.41**	**19.47**	**0.30**	**1.28**
3	**28.26**	**1.73**	**28.26**	**0.47**	**1.45**
4	**34.74**	**2**	**34.74**	**0.60**	**1.54**
5	**41**	**2.23**	**41**	**0.69**	**1.61**
6	**46.37**	**2.44**	**46.37**	**0.77**	**1.66**
7	**50.17**	**2.64**	**50.17**	**0.84**	**1.70**
8	**56.17**	**2.82**	**56.17**	**0.90**	**1.74**
9	**61.04**	**3**	**61.04**	**0.95**	**1.78**
10	**67.28**	**3.16**	**67.28**	**1**	**1.82**
12	**72.87**	**3.46**	**72.87**	**1.07**	**1.86**
24	**97.03**	**4.89**	**97.03**	**1.38**	**1.98**

n = 3

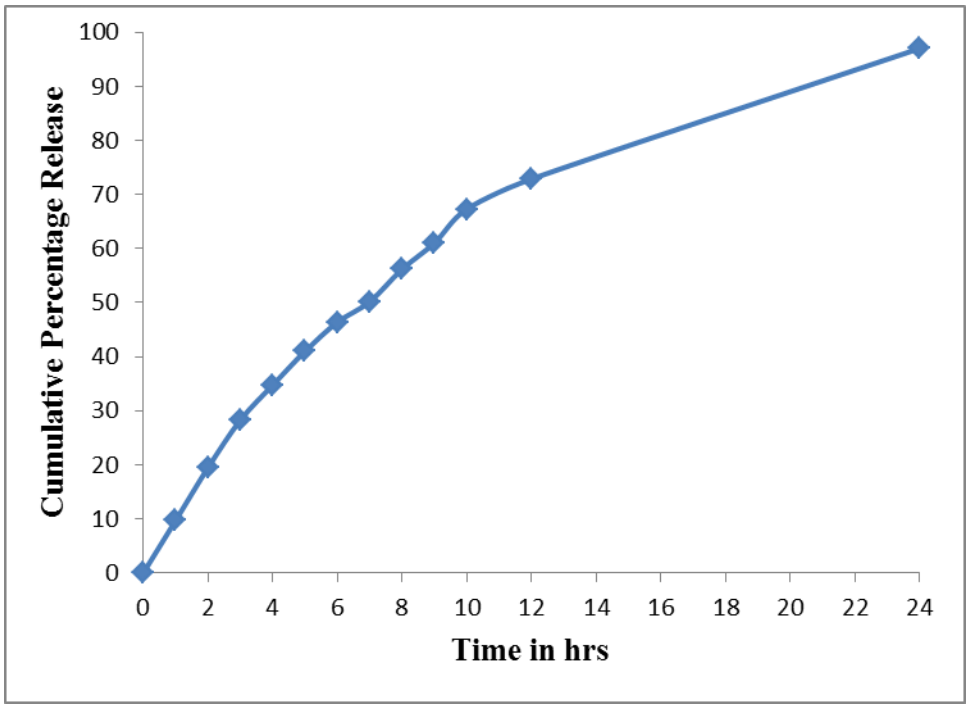


Fig.19: Zero order Release Plot for Formulation PXN-2

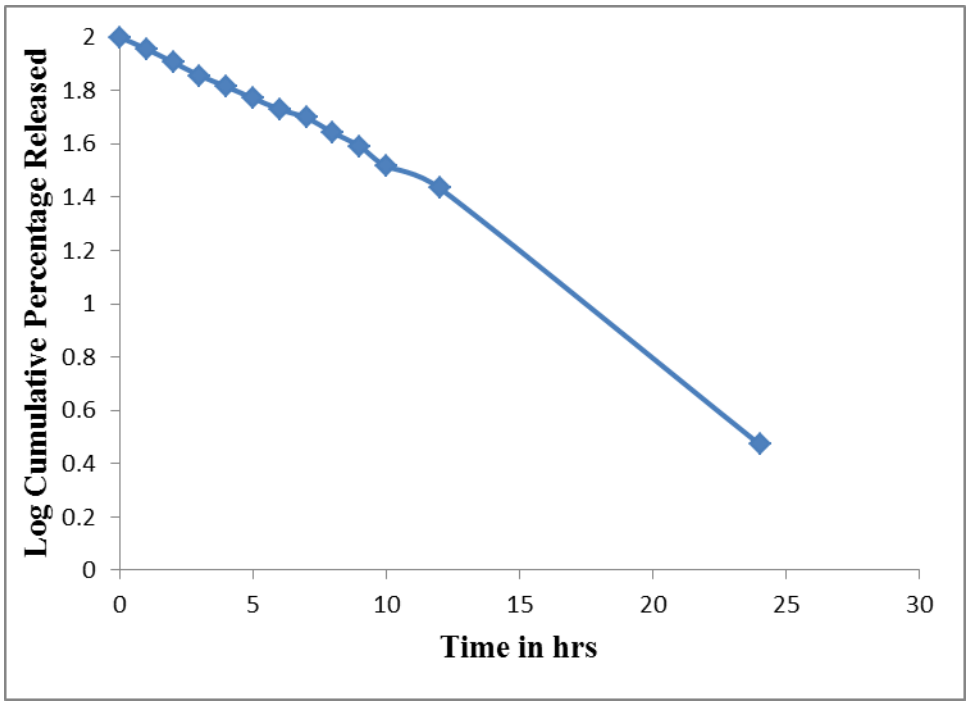


Fig.20: First order Release Plot for Formulation PXN-2

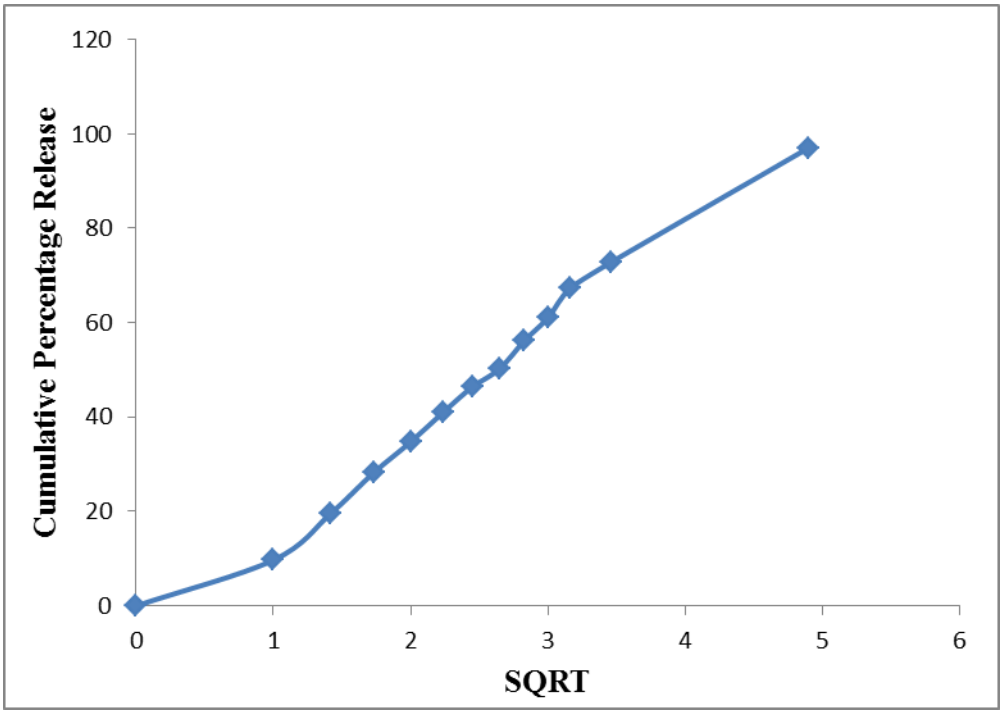


Fig.21: Higuchis Release Plot for Formulation PXN-2

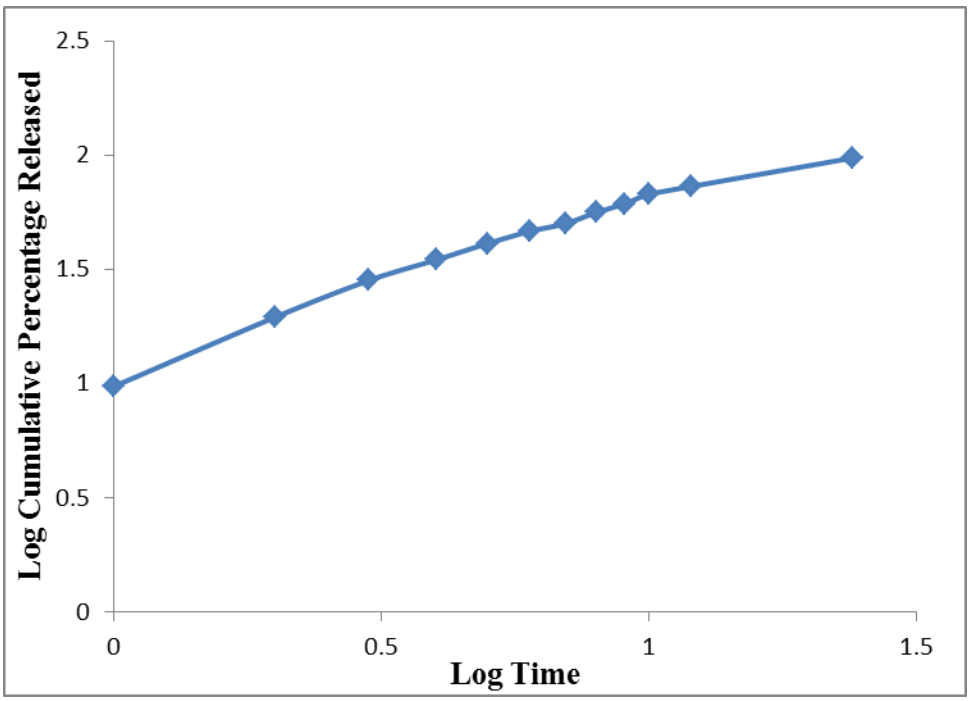


Fig.22: Peppas Release Plot for Formulation PXN-2

Table 22: *Invitro* Release Profile of Paclitaxel Nanoparticle from Formulation PXN-3

***In vitro* drug release data**		**Higuchi's data**		**Peppa's data**	
Time (hrs)	**Cumulative % drug release**	**Square root time**	**Cumulative % drug release**	**Log time**	**Log cumulative % drug release**
1	**8.12**	**1**	**8.12**	**0**	**0.90**
2	**17.30**	**1.41**	**17.30**	**0.30**	**1.23**
3	**26.27**	**1.73**	**26.27**	**0.47**	**1.41**
4	**33.13**	**2**	**33.13**	**0.60**	**1.52**
5	**39.01**	**2.23**	**39.01**	**0.69**	**1.59**
6	**43.85**	**2.44**	**43.85**	**0.77**	**1.64**
7	**48.89**	**2.64**	**48.89**	**0.84**	**1.68**
8	**54.31**	**2.82**	**54.31**	**0.90**	**1.73**
9	**58.06**	**3**	**58.06**	**0.95**	**1.76**
10	**64.75**	**3.16**	**64.75**	**1**	**1.81**
12	**69.24**	**3.46**	**69.24**	**1.07**	**1.84**
24	**95.25**	**4.89**	**95.25**	**1.38**	**1.97**

n = 3

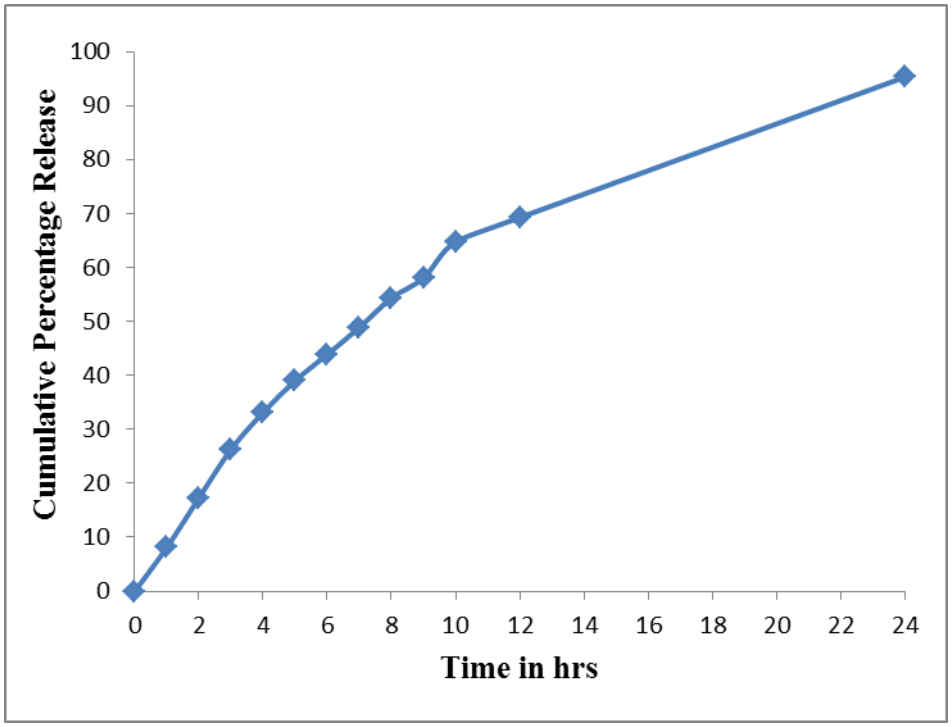


Fig .23: Zero Order Release Plot for Formulationpaclitaxel-3

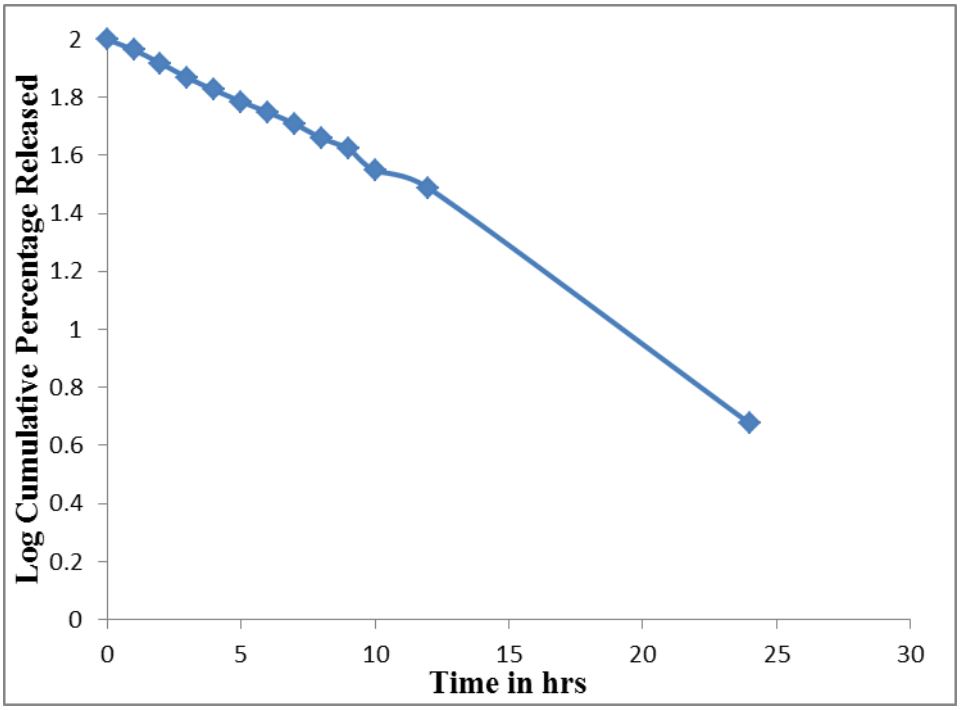


Fig.24: First order Release Plot for Formulation PXN-3

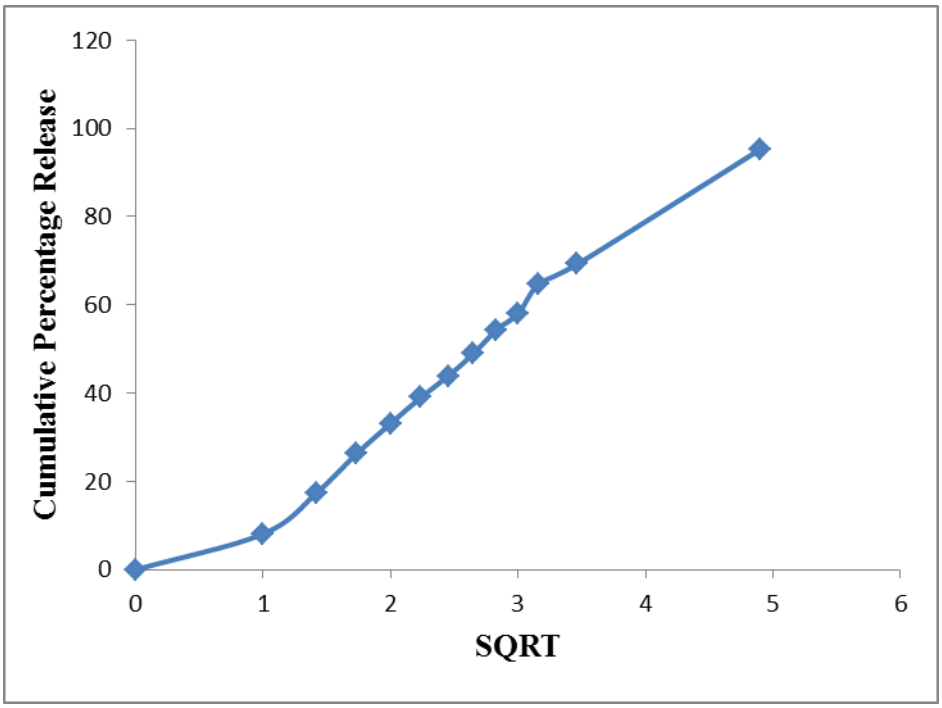


Fig.25: Higuchis Release Plot for Formulation PXN-3

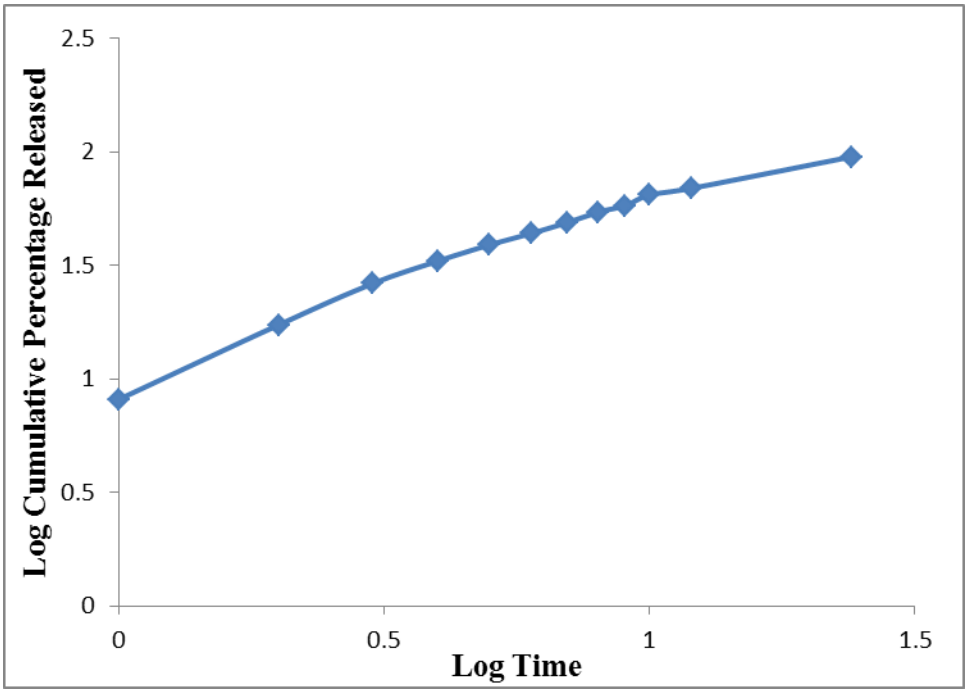


Fig.26: Peppas Release Plot for Formulation PXN-3

Table 23: *Invitro* Release Profile of Paclitaxel Nanoparticle from Formulation PXN-4

***In vitro* drug release data**		**Higuchi's data**		**Peppa's data**	
Time (hrs)	**Cumulative % drug release**	**Square root time**	**Cumulative % drug release**	**Log time**	**Log cumulative % drug release**
1	**8.52**	**1**	**8.52**	**0**	**0.93**
2	**18.68**	**1.41**	**18.68**	**0.30**	**1.27**
3	**26.25**	**1.73**	**26.25**	**0.47**	**1.4**
4	**33.50**	**2**	**33.50**	**0.60**	**1.52**
5	**39.06**	**2.23**	**39.06**	**0.69**	**1.59**
6	**43.81**	**2.44**	**43.81**	**0.77**	**1.64**
7	**48.45**	**2.64**	**48.45**	**0.84**	**1.68**
8	**54.14**	**2.82**	**54.14**	**0.90**	**1.73**
9	**59.9**	**3**	**59.97**	**0.95**	**1.77**
10	**63.24**	**3.162**	**63.24**	**1**	**1.80**
12	**69.17**	**3.46**	**69.17**	**1.07**	**1.83**
24	**97.47**	**4.89**	**97.47**	**1.38**	**1.98**

n = 3

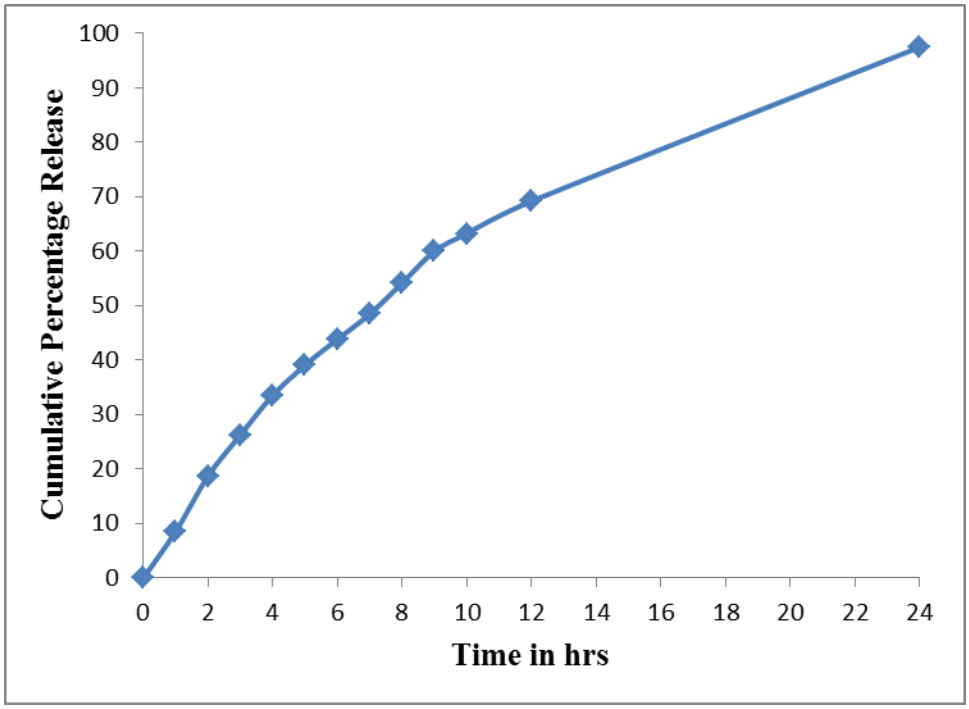


Fig.27: Zero Order Release Plot for Formulation paclitaxel-4 Nanoparticles

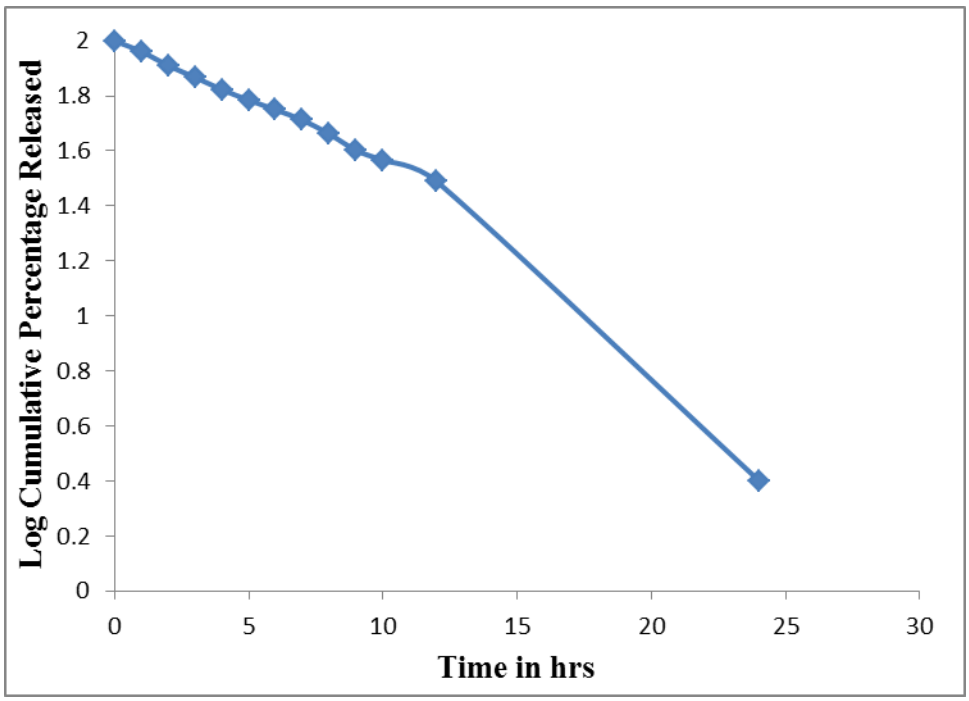


Fig.28: First order Release Plot for Formulation PXN-4

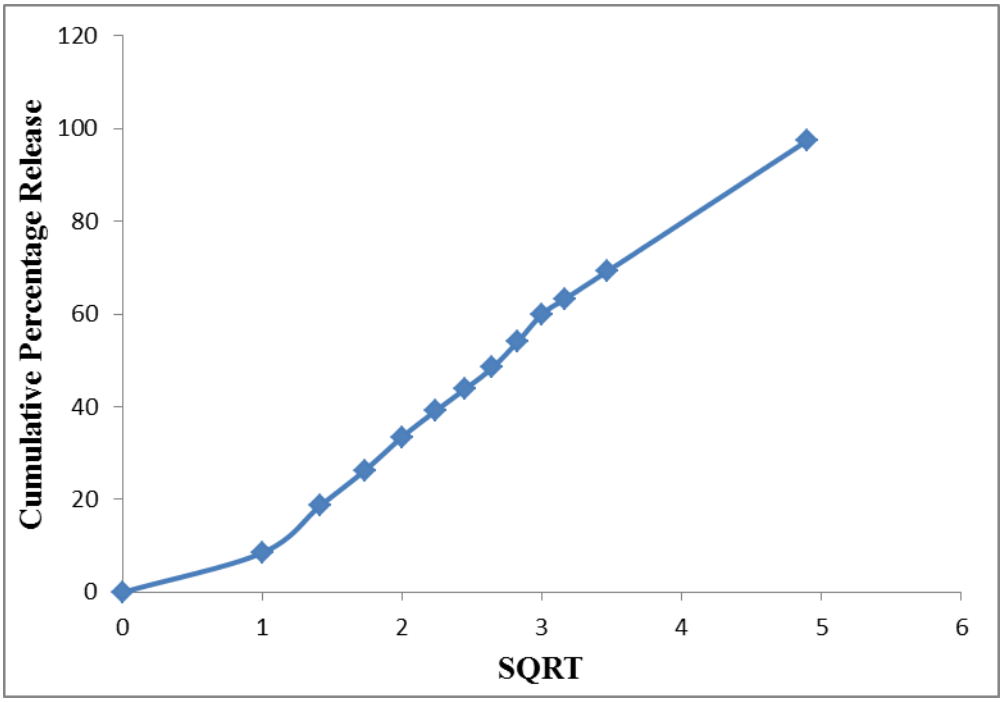


Fig.29: Higuchis Release Plot for Formulation paclitaxel-4 Nanoparticles

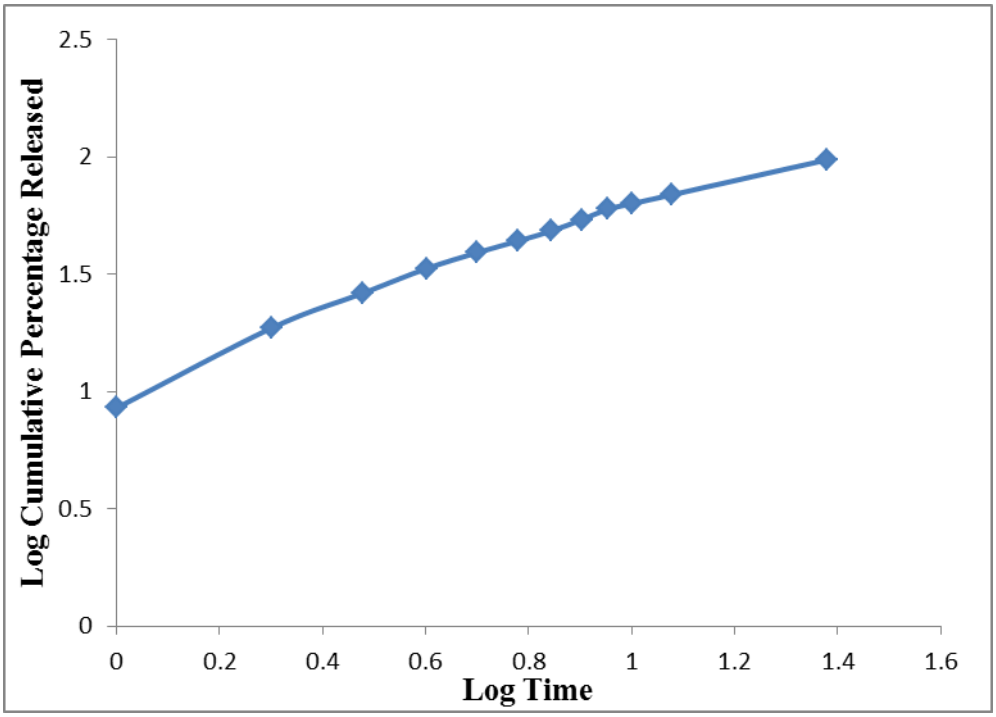


Fig .30: Peppas Release Plot for Formulation PXN-4

Table 24: *Invitro* Release Profile of Paclitaxel Nanoparticle from Formulation PXN-5

***In vitro* drug release data**		**Higuchi's data**		**Peppa's data**	
Time (hrs)	**Cumulative % drug release**	**Square root time**	**Cumulative % drug release**	**Log time**	**Log cumulative % drug release**
1	**6.79**	**1**	**6.79**	**0**	**0.83**
2	**16.76**	**1.41**	**16.76**	**0.30**	**1.22**
3	**24.57**	**1.73**	**24.57**	**0.47**	**1.39**
4	**31.22**	**2**	**31.22**	**0.60**	**1.49**
5	**37.02**	**2.23**	**37.02**	**0.69**	**1.56**
6	**42.24**	**2.44**	**42.24**	**0.77**	**1.62**
7	**46.78**	**2.64**	**46.78**	**0.84**	**1.67**
8	**52.33**	**2.82**	**52.33**	**0.90**	**1.71**
9	**57.87**	**3**	**57.87**	**0.95**	**1.76**
10	**61.34**	**3.16**	**61.34**	**1**	**1.78**
12	**67.10**	**3.46**	**67.10**	**1.07**	**1.82**
24	**94.60**	**4.89**	**94.60**	**1.38**	**1.97**

n = 3

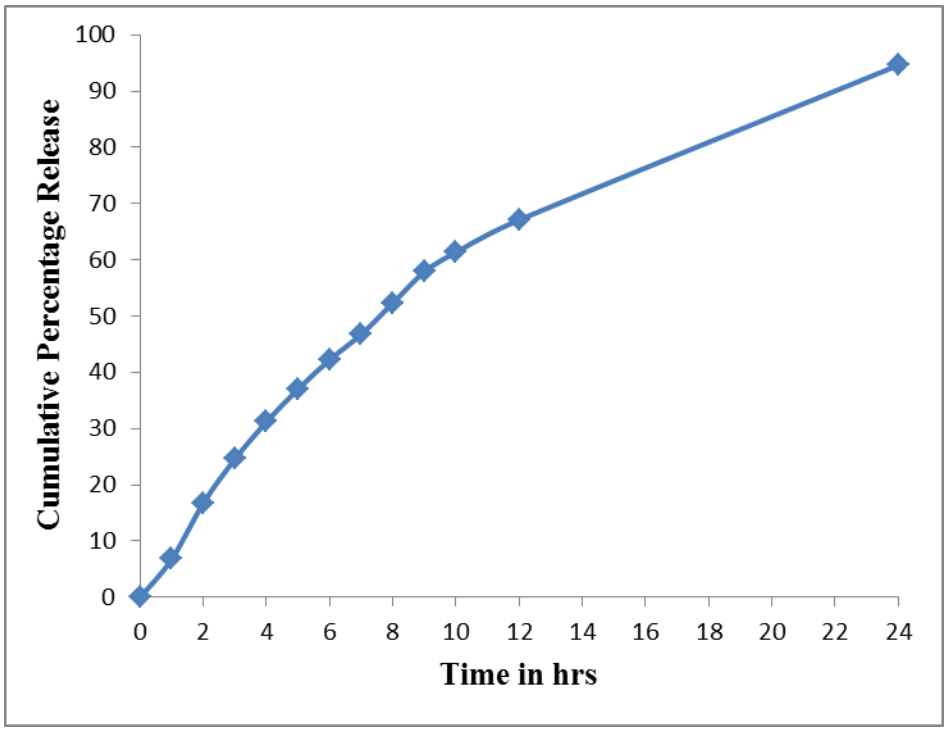


Fig. 31: Zero Order Release Plot for Formulation PXN-5

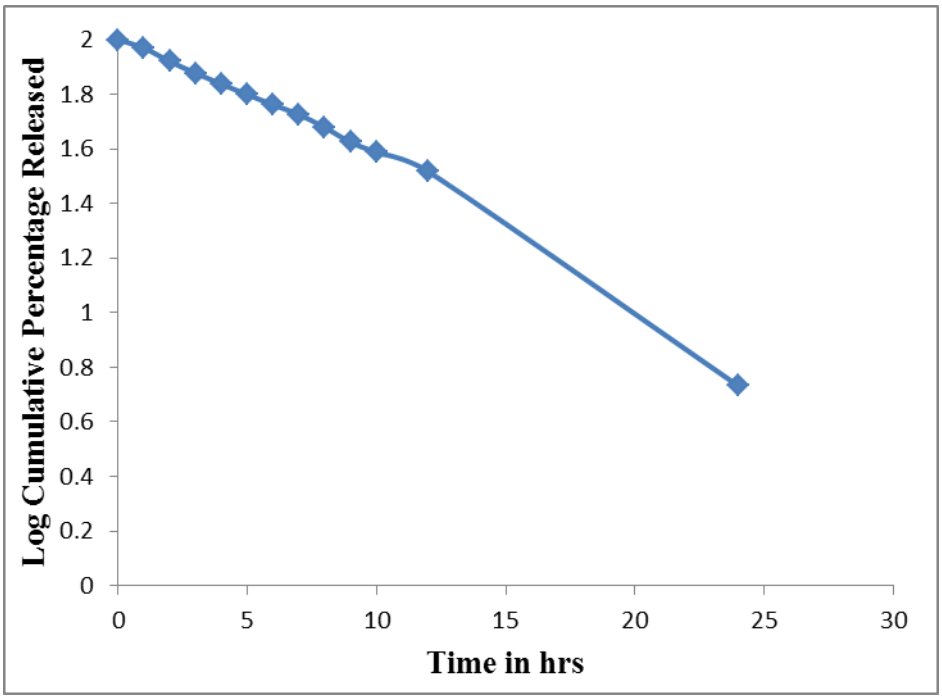


Fig. 32: First order Release Plot for Formulation PXN-5

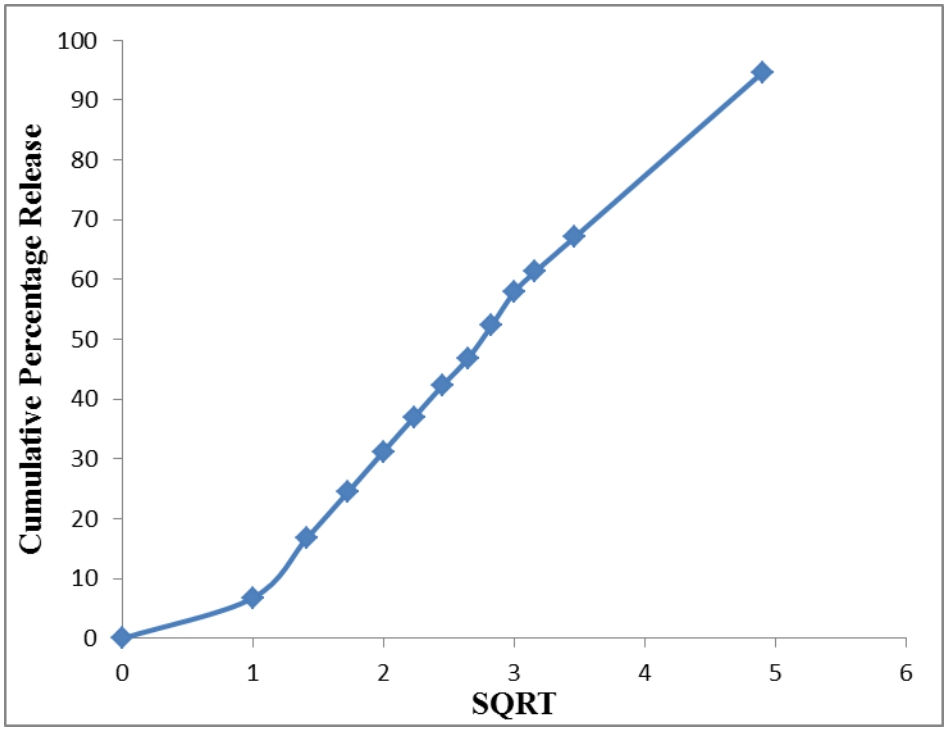


Fig.33: Higuchis Release Plot for Formulation PXN-5

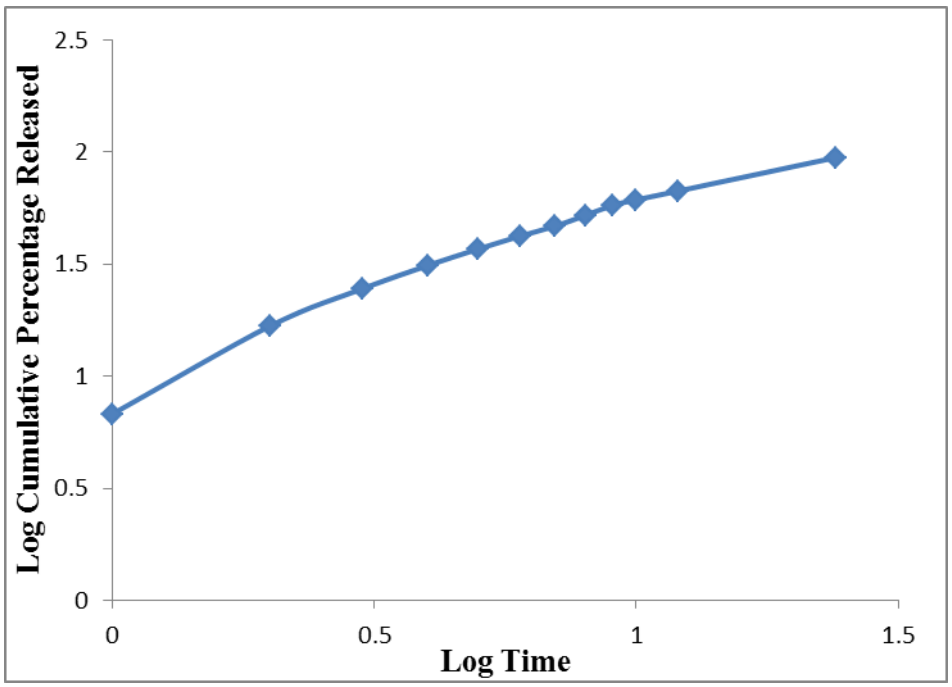


Fig .34: Peppas Release Plot for Formulation PXN-5

Table 25: *Invitro* Release Profile of Paclitaxel Nanoparticle from Formulation PXN-6

***In vitro* drug release data**		**Higuchi's data**		**Peppa's data**	
Time (hrs)	**Cumulative % drug release**	**Square root time**	**Cumulative % drug release**	**Log time**	**Log cumulative % drug release**
1	**4.90**	**1**	**4.90**	**0**	**0.69**
2	**12.98**	**1.41**	**12.98**	**0.30**	**1.11**
3	**20.80**	**1.73**	**20.80**	**0.47**	**1.31**
4	**25.84**	**2**	**25.84**	**0.60**	**1.41**
5	**30.74**	**2.23**	**30.74**	**0.69**	**1.48**
6	**34.82**	**2.44**	**34.82**	**0.77**	**1.54**
7	**40.59**	**2.64**	**40.59**	**0.84**	**1.60**
8	**45.02**	**2.82**	**45.02**	**0.90**	**1.65**
9	**51.92**	**3**	**51.92**	**0.95**	**1.71**
10	**57.96**	**3.16**	**57.96**	**1**	**1.76**
12	**63.68**	**3.46**	**63.68**	**1.07**	**1.80**
24	**92.85**	**4.89**	**92.85**	**1.38**	**1.96**

n = 3

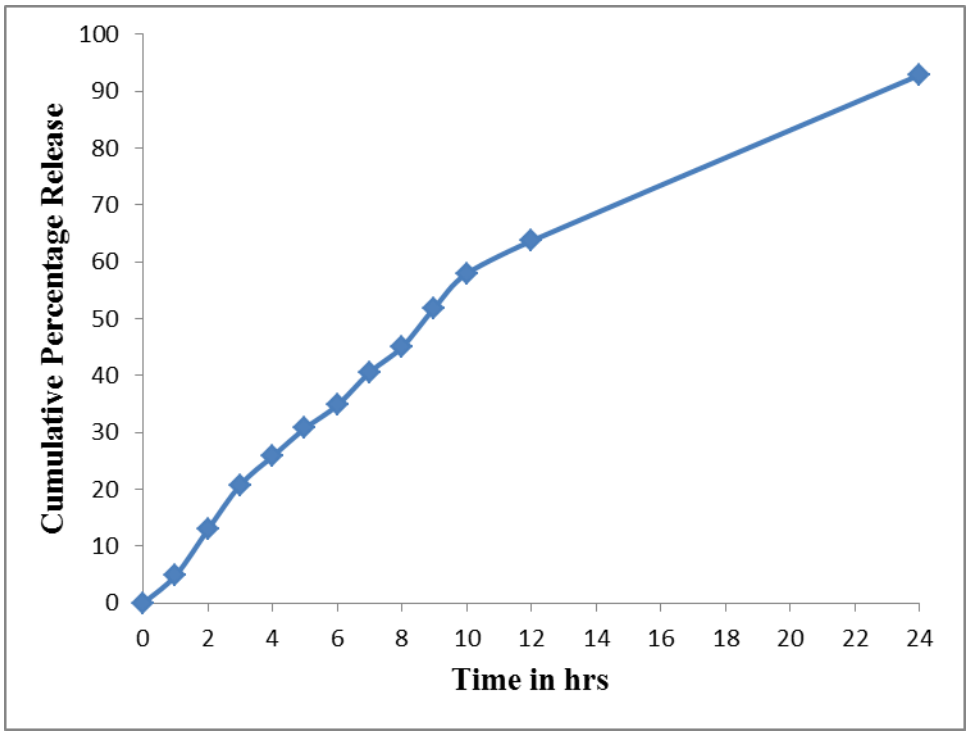


Fig.35:Zero order Release Plot for Formulation PXN-6

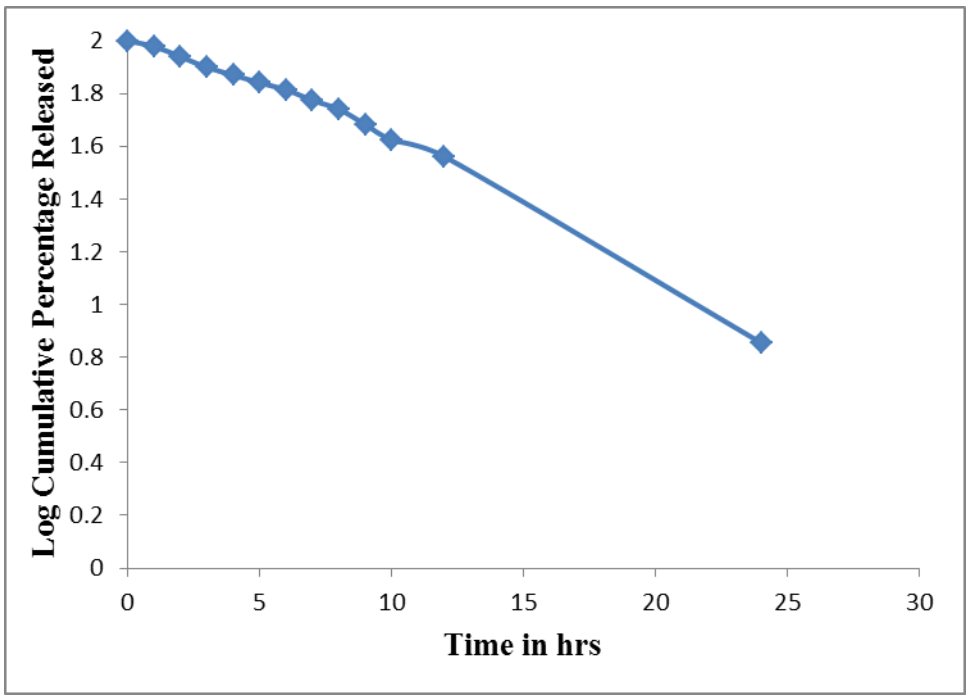


Fig. 36: First order Release Plot for Formulation PXN-6

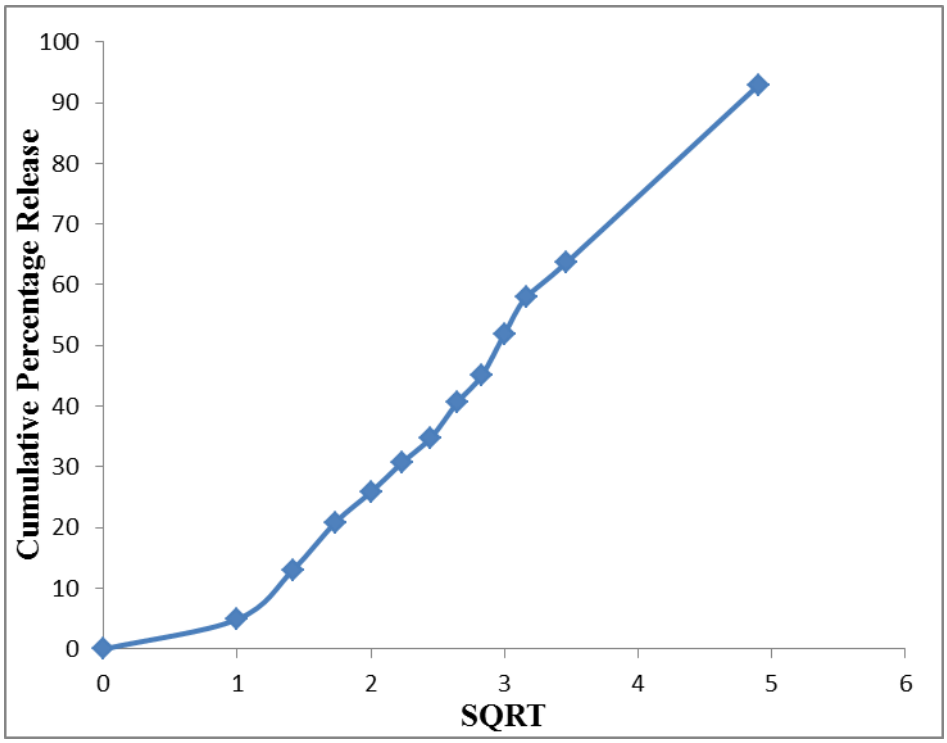


Fig .37: Higuchis Release Plot for Formulation PXN-6

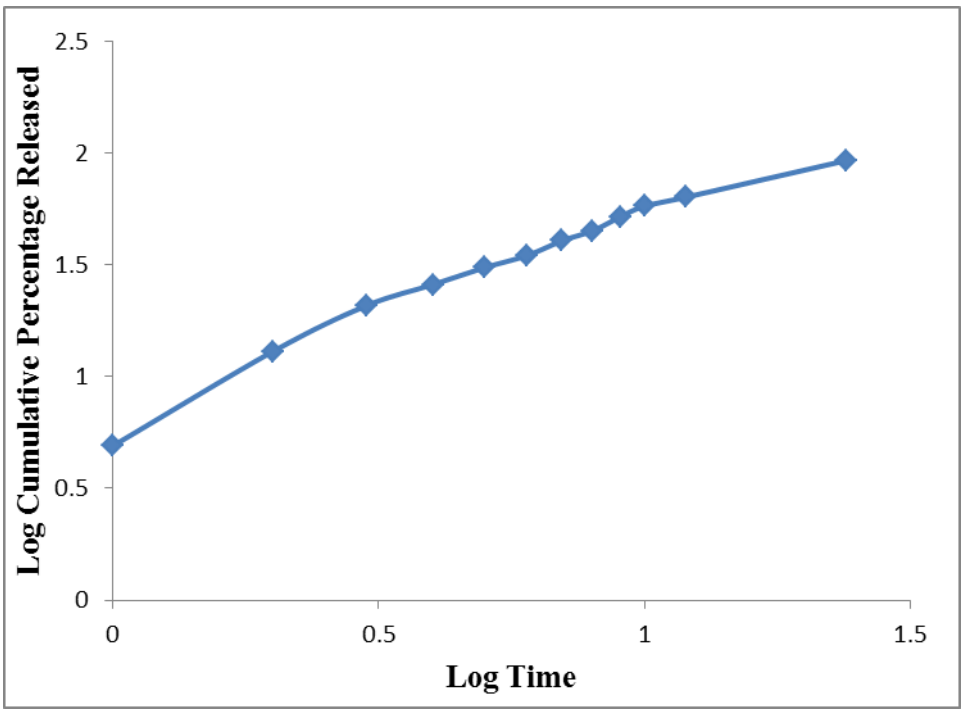


Fig.38: Peppas Release Plot for Formulation PXN-6

Table 26: *Invitro* Release Profile of Paclitaxel Nanoparticle from Formulation PXN-7

***In vitro* drug release data**		**Higuchi's data**		**Peppa's data**	
Time (hrs)	**Cumulative % drug release**	**Square root time**	**Cumulative % drug release**	**Log time**	**Log cumulative % drug release**
1	**12.09**	**1**	**12.15**	**0**	**1.08**
2	**21.73**	**1.46**	**21.69**	**0.35**	**1.31**
3	**24.85**	**1.78**	**29.80**	**0.49**	**1.47**
4	**32.02**	**2**	**36.07**	**0.64**	**1.58**
5	**42.20**	**2.28**	**45.22**	**0.66**	**1.65**
6	**51.51**	**2.46**	**52.53**	**0.75**	**1.75**
7	**53.00**	**2.68**	**56**	**0.87**	**1.74**
8	**56.32**	**2.72**	**59.36**	**0.93**	**1.77**
9	**64.89**	**3**	**64.90**	**0.94**	**1.82**
10	**68.27**	**3.20**	**69.24**	**1**	**1.86**
12	**76.70**	**3.32**	**74.64**	**1.09**	**1.82**
24	**88.00**	**4.85**	**99.00**	**1.36**	**1.94**

n = 3

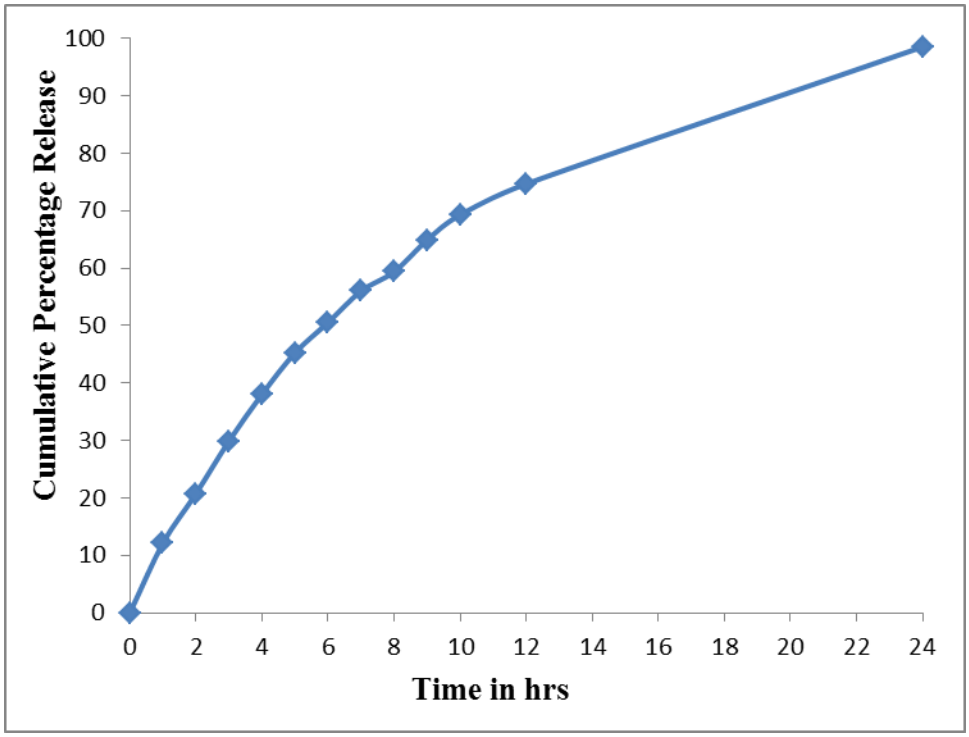


Fig.15: Zero Order Release Plot for Formulation PXN-7

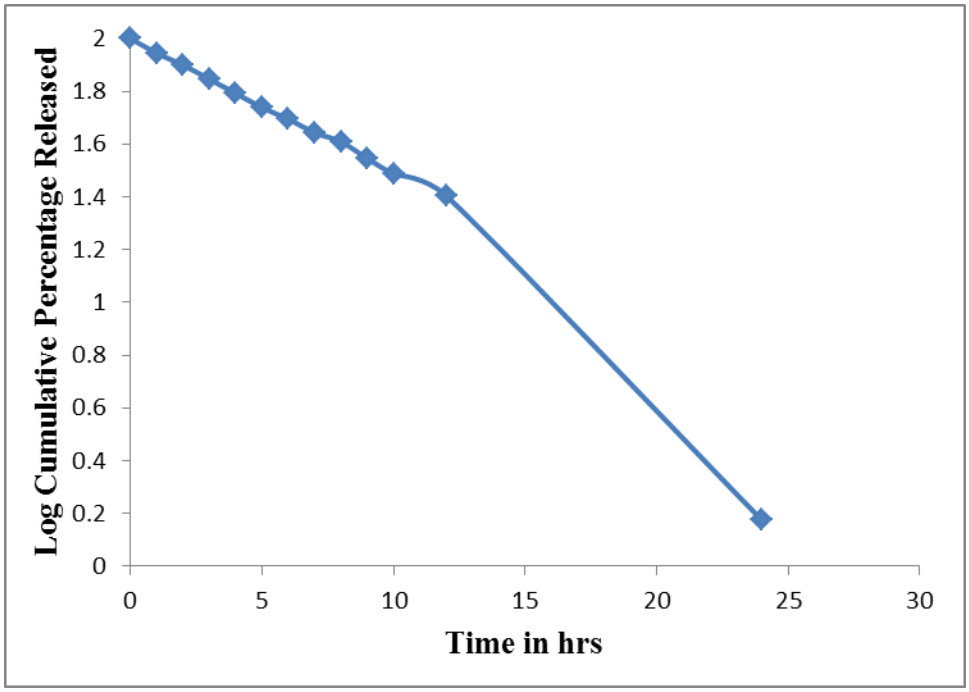


Fig.16: First order Release Plot for Formulation PXN-7

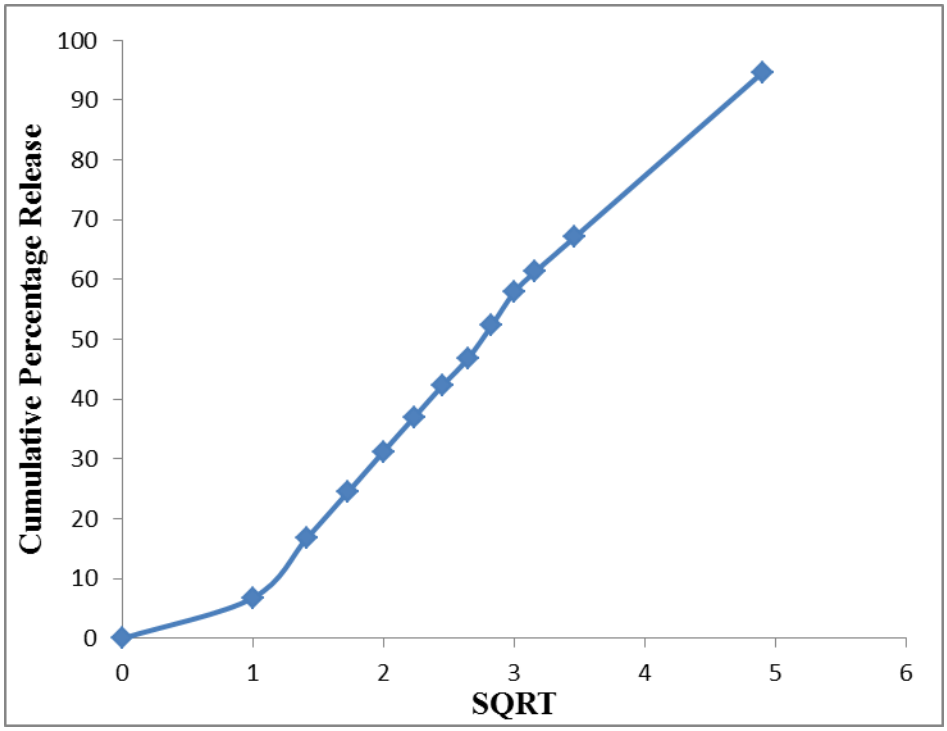


Fig.17: Higuchi Release Plot for Formulation PXN-7

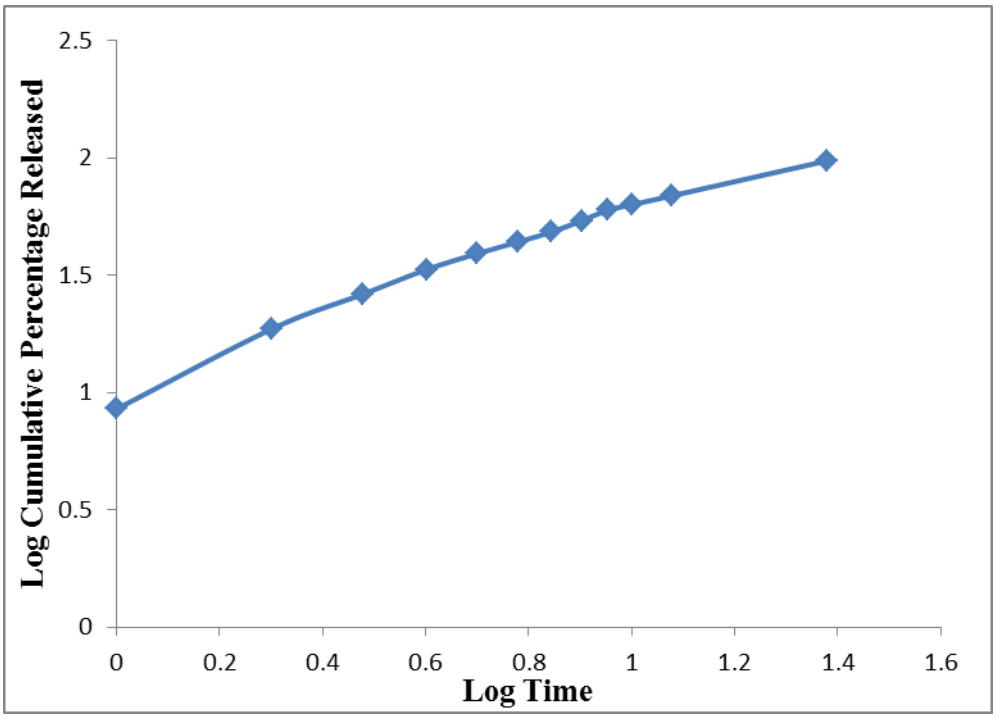


Fig.18: Peppas Release Plot for Formulation PXN-7

Table 27: *Invitro* Release Profile of Paclitaxel Nanoparticle from Formulation PXN-8

***In vitro* drug release data**		**Higuchi's data**		**Peppa's data**	
Time (hrs)	**Cumulative % drug release**	**Square root time**	**Cumulative % drug release**	**Log time**	**Log cumulative % drug release**
1	**9.60**	**1**	**9.62**	**0**	**0.93**
2	**15.40**	**1.44**	**19.40**	**0.30**	**1.24**
3	**29.23**	**1.66**	**28.22**	**0.42**	**1.42**
4	**32.69**	**2**	**32.70**	**0.62**	**1.53**
5	**45**	**2.44**	**45**	**0.63**	**1.60**
6	**45.30**	**2.45**	**42.32**	**0.72**	**1.63**
7	**52.13**	**2.72**	**51.16**	**0.81**	**1.72**
8	**53.15**	**2.80**	**56.18**	**0.93**	**1.70**
9	**63.02**	**3**	**64.07**	**0.92**	**1.73**
10	**64.38**	**3.12**	**63.22**	**1**	**1.80**
12	**70.83**	**3.40**	**75.87**	**1.03**	**1.83**
24	**97.00**	**4.80**	**99.03**	**1.32**	**1.93**

n = 3

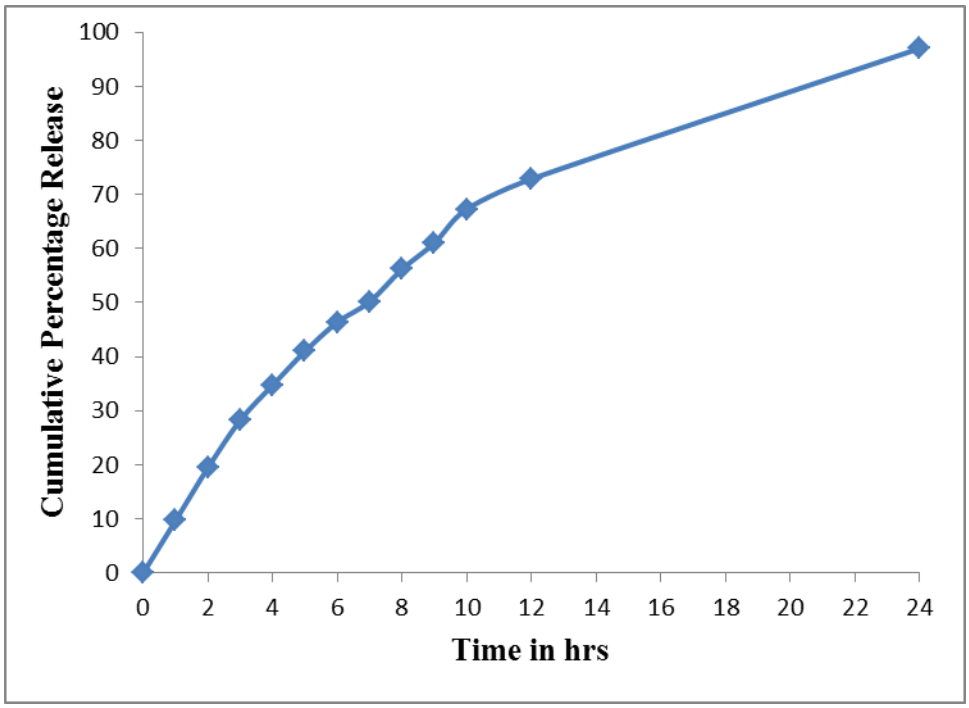


Fig.19: Zero order Release Plot for Formulation PXN-8

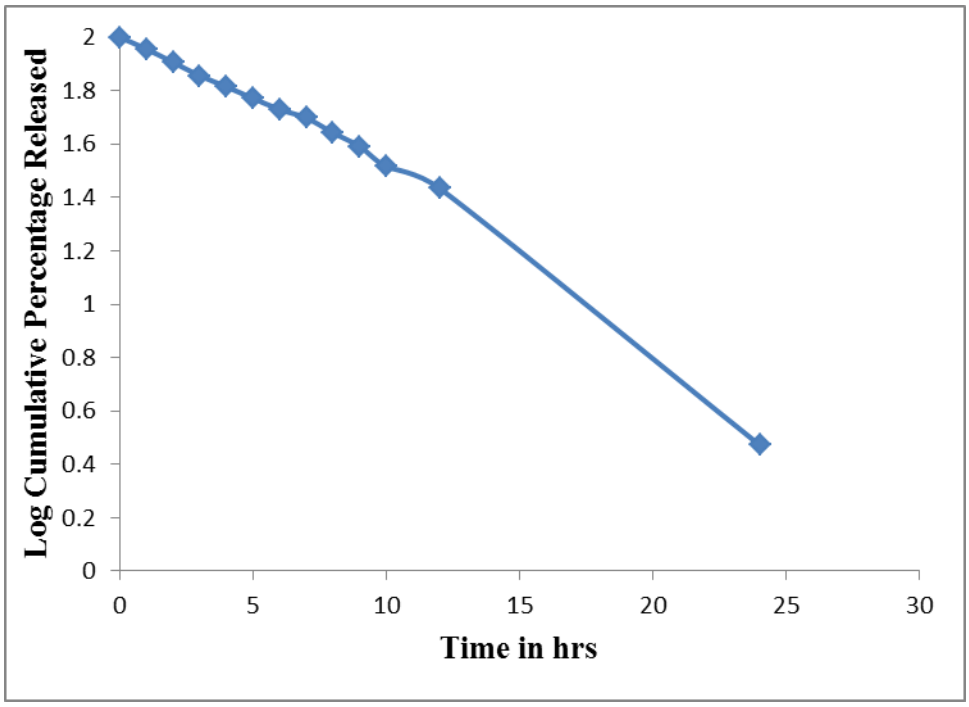


Fig.20: First order Release Plot for Formulation PXN-8

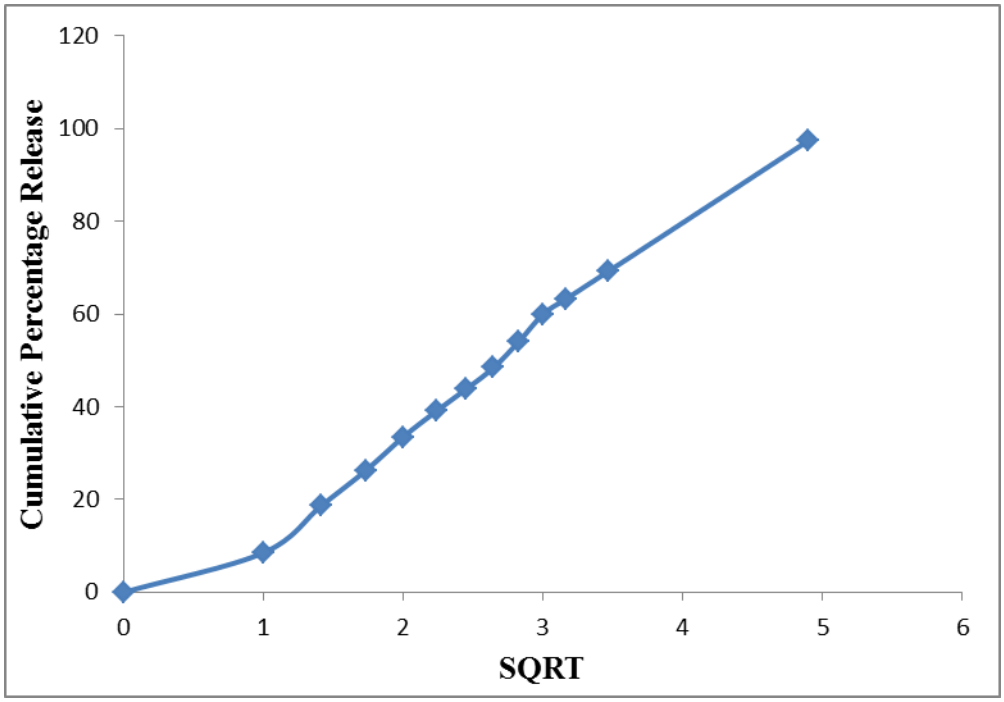


Fig.21: Higuchis Release Plot for Formulation PXN-8

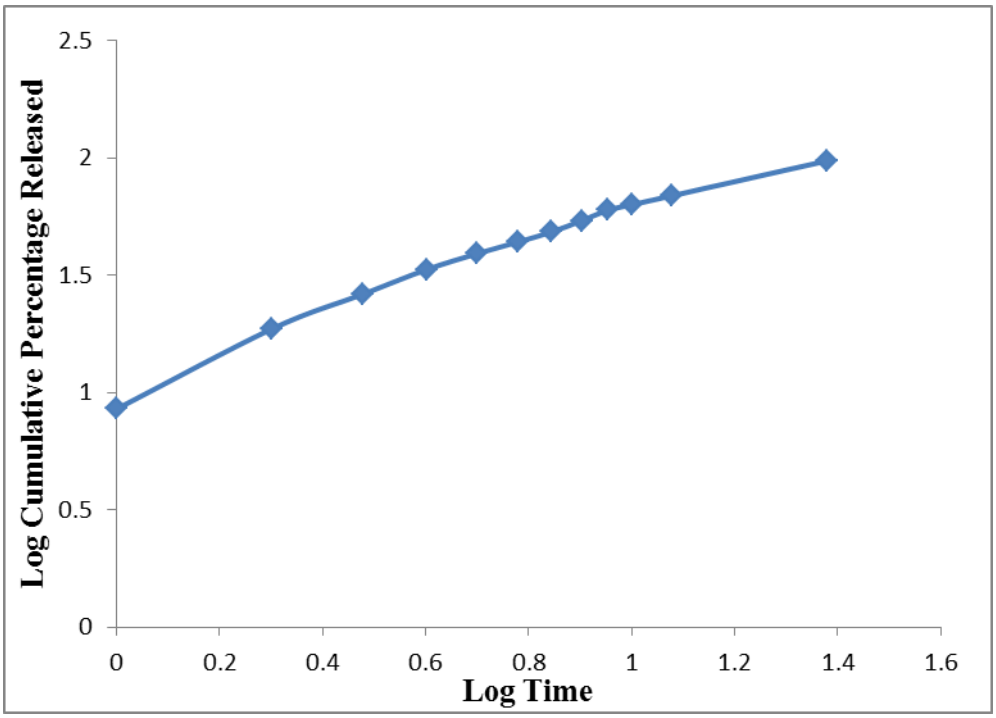


Fig.22: Peppas Release Plot for Formulation PXN-8

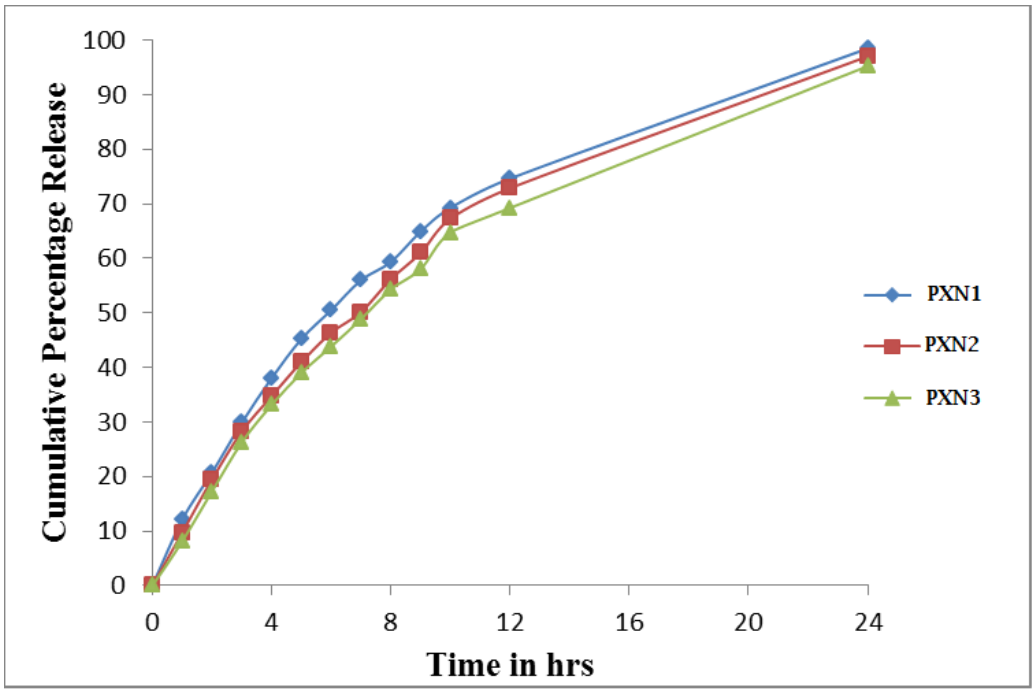


Fig.39: Comparative Zero order Release Plot for Formulations PXN-1 to PXN-3 Nanoparticles

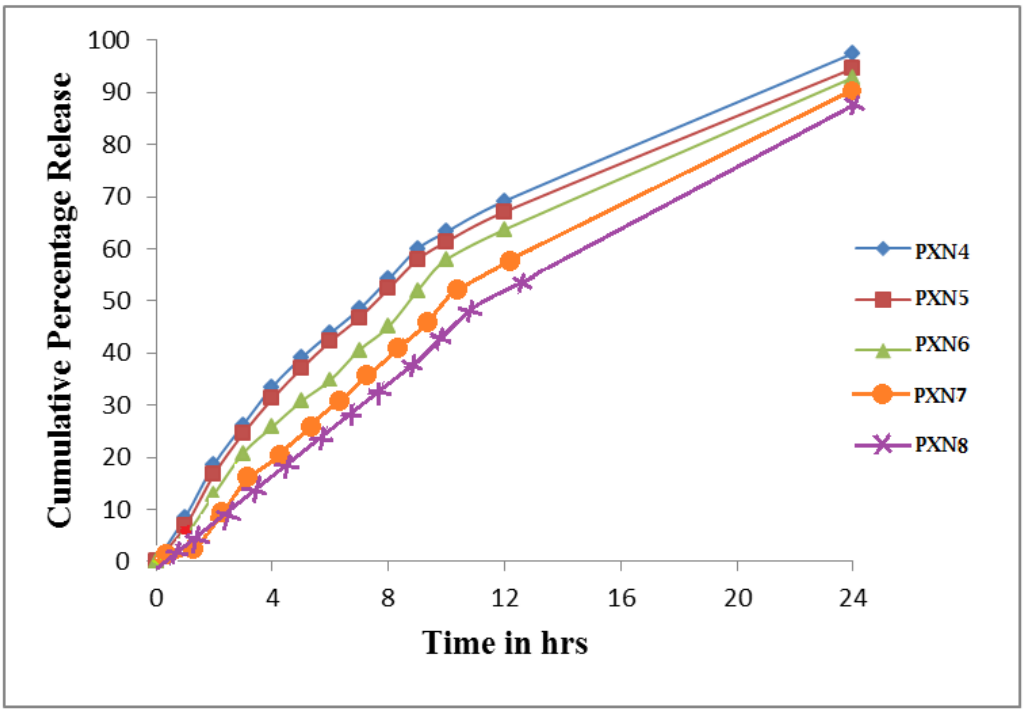


Fig .40: Comparative Zero order Release Plot for Formulations PXN-4 to PXN-8 Nanoparticles

Table 28 :Release Profile of Paclitaxel NP From Formulation PXN-1 to PXN-8

Time (hrs)	Cumalative % Drug rlease							
	PXN-1	PXN-2	PXN-3	PXN-4	PXN-5	PXN-6	PXN-7	PXN-8
1	12.09	9.69	8.12	8.52	6.79	4.90	12.09	9.60
2	20.73	19.47	17.30	18.68	16.76	12.98	21.73	15.40
3	29.87	28.26	26.27	26.25	24.57	20.80	24.85	29.23
4	38.04	34.74	33.13	33.50	31.22	25.84	32.02	32.69
5	45.22	41	39.01	39.06	37.02	30.74	42.20	45
6	50.53	46.37	43.85	43.81	42.24	34.82	51.51	45.30
7	56.00	50.17	48.89	48.45	46.78	40.59	53.00	52.13
8	59.36	56.17	54.31	54.14	52.33	45.02	56.32	53.15
9	64.90	61.04	58.06	59.9	57.87	51.92	64.89	63.02
10	69.24	67.28	64.75	63.24	61.34	57.96	68.27	64.38
12	74.64	72.87	69.24	69.17	67.10	63.68	76.70	70.83
24	98.50	97.03	95.25	97.47	94.60	92.85	88.00	97.00

n = 3

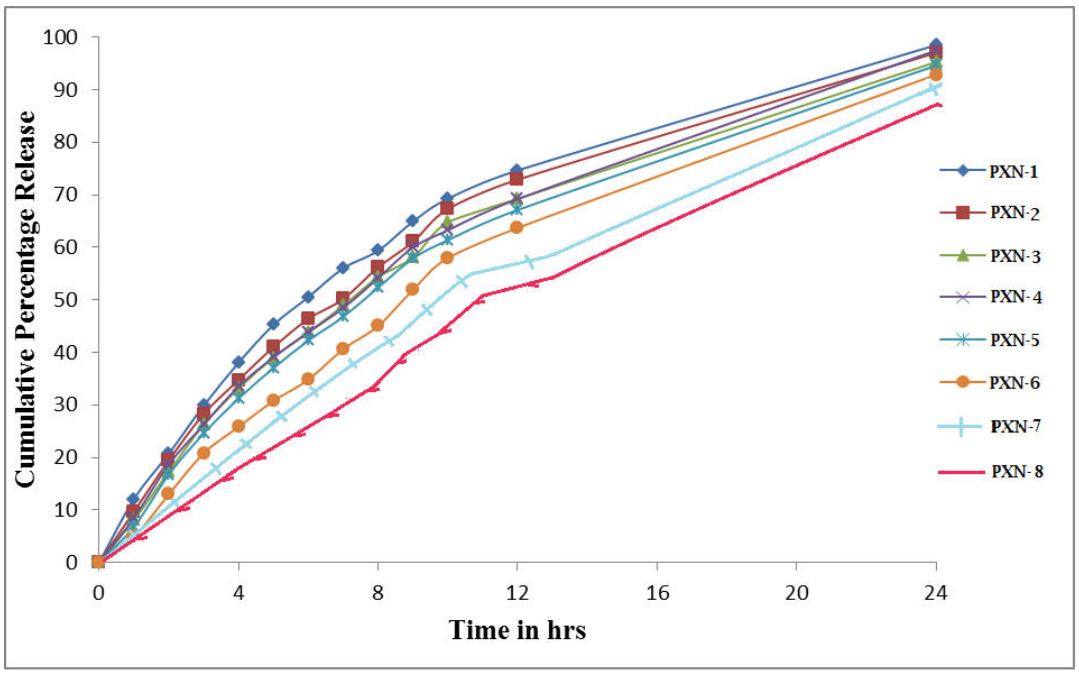


Fig. 41: Comparative Zero order Release Plot for Formulations PXN-1to PXN-8

Table 29*: In-Vitro* Release Kinetics of Prepared Nanoparticles

Formulation code	Mathematical models (release kinetics)				
	Zero order kinetics	First order kinetics	Higuchi's	Peppa's	
	r^2	r^2	r^2	r^2	N
PXN -1	0.86	0.95	0.98	0.97	0.68
PXN -2	0.88	0.97	0.98	0.97	0.73
PXN -3	0.89	0.98	0.98	0.97	0.78
PXN -4	0.91	0.94	0.99	0.97	0.76
PXN -5	0.90	0.97	0.99	0.99	0.82
PXN -6	0.93	0.97	0.99	0.97	0.91
PXN -7	0.88	0.92	0.99	0.97	0.65
PXN -8	0.90	0.96	0.99	0.97	0.72

Table 30: Drug entrapment efficiency of loaded Nanoparticles

S.No	Formulation code	Quantity of $AgNo_3$	Theoretical drug loading (%)	Actual drug loading ±S.D	Drug Entrapment Efficiency (%)	Particle Size
1	**PXN-1**	0.017gm	89.5	86.51	96.66	1.51µm
2	**PXN-2**	0.17gm	46.8	44.9	96	1.26µm
3	**PXN-3**	0.5gm	23.07	21.98	95.3	1.03µm
4	**PXN-4**	0.8 gm	15.7	14.85	94.6	9.27nm
5	**PXN-5**	1.0 gm	13.04	12.25	94	856nm
6	**PXN-6**	**1.17gm**	**11.36**	**10.59**	**93.3**	**717nm**
7	**PXN-7**	1.17gm	11.36	10.50	92.4	635nm
8	**PXN-8**	1.17gm	11.36	10.20	89.78	935nm

7.6 Particle Size Analysis (PSA)(Optmized Formulation PXN-6)

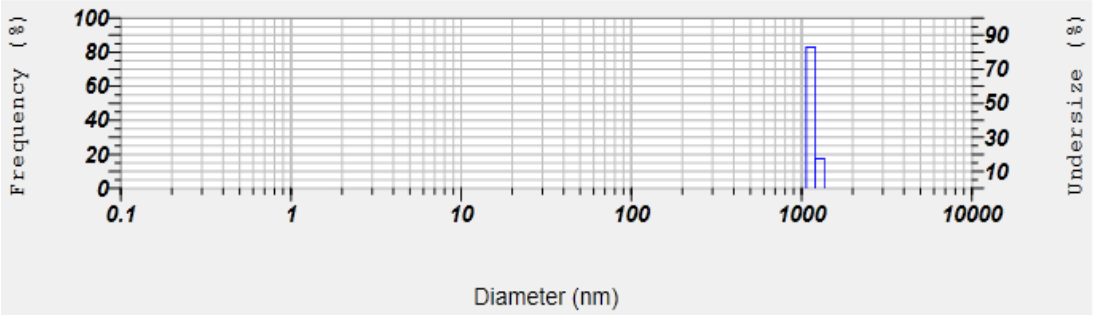


Fig.42:Particle Size Analysis of paclitaxel silver nitrate nanoparticles

20170418161500l.nsz
Measurement Results

Date	:	18 April 2017 16:15:25
Measurement Type	:	Particle Size
Sample Name	:	PaclitaxelNanoParticals-Size
Scattering Angle	:	90
Temperature of the holder	:	25.0 deg. C
T% before meas.	:	22350
Viscosity of the dispersion medium	:	2.035 mPa.s
Form Of Distribution	:	|Standard|
Representation of result	:	Scattering Light Intensity
Count rate	:	1311 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	1161.3 nm	55.8 nm	1150.4 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	1161.3 nm	55.8 nm	1150.4 nm

Histogram Operations

Size (Median)	:	1150.4 nm
Mode	:	1150.4 nm
% Cumulative (1)	:	10.0 (%) - 1084.4 (nm)
% Cumulative (2)	:	50.0 (%) - 1150.4 (nm)
% Cumulative (3)	:	90.0 (%) - 1271.1 (nm)
% Cumulative (4)	:	30.0 (%) - 1116.9 (nm)
% Cumulative (5)	:	40.0 (%) - 1133.5 (nm)
% Cumulative (6)	:	50.0 (%) - 1150.4 (nm)
% Cumulative (7)	:	20.0 (%) - 1100.5 (nm)
% Cumulative (8)	:	70.0 (%) - 1184.8 (nm)
% Cumulative (9)	:	95.0 (%) - 1316.7 (nm)
% Cumulative (10)	:	100.0 (%) - 8510.6 (nm)

Cumulant Operations

Z-Average	:	717.4 nm
PI	:	2.235

Particle Size Report of Silver Nanoparticle PXN

No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	22	4.40	0.000	0.000	43	57.09	0.000	0.000	64	740.89	0.000	0.000
2	0.38	0.000	0.000	23	4.97	0.000	0.000	44	64.50	0.000	0.000	65	837.07	0.000	0.000
3	0.43	0.000	0.000	24	5.61	0.000	0.000	45	72.87	0.000	0.000	66	945.74	0.000	0.000
4	0.49	0.000	0.000	25	6.34	0.000	0.000	46	82.33	0.000	0.000	67	1068.52	0.000	0.000
5	0.55	0.000	0.000	26	7.17	0.000	0.000	47	93.02	0.000	0.000	68	1207.24	82.695	82.695
6	0.62	0.000	0.000	27	8.10	0.000	0.000	48	105.10	0.000	0.000	69	1363.97	17.305	100.000
7	0.70	0.000	0.000	28	9.15	0.000	0.000	49	118.74	0.000	0.000	70	1541.04	0.000	100.000
8	0.80	0.000	0.000	29	10.34	0.000	0.000	50	134.16	0.000	0.000	71	1741.10	0.000	100.000
9	0.90	0.000	0.000	30	11.68	0.000	0.000	51	151.57	0.000	0.000	72	1967.14	0.000	100.000
10	1.02	0.000	0.000	31	13.20	0.000	0.000	52	171.25	0.000	0.000	73	2222.51	0.000	100.000
11	1.15	0.000	0.000	32	14.91	0.000	0.000	53	193.48	0.000	0.000	74	2511.05	0.000	100.000
12	1.30	0.000	0.000	33	16.84	0.000	0.000	54	218.60	0.000	0.000	75	2837.04	0.000	100.000
13	1.47	0.000	0.000	34	19.03	0.000	0.000	55	246.98	0.000	0.000	76	3205.35	0.000	100.000
14	1.66	0.000	0.000	35	21.50	0.000	0.000	56	279.04	0.000	0.000	77	3621.45	0.000	100.000
15	1.87	0.000	0.000	36	24.29	0.000	0.000	57	315.27	0.000	0.000	78	4091.63	0.000	100.000
16	2.11	0.000	0.000	37	27.45	0.000	0.000	58	356.20	0.000	0.000	79	4622.81	0.000	100.000
17	2.39	0.000	0.000	38	31.01	0.000	0.000	59	402.44	0.000	0.000	80	5222.45	0.000	100.000
18	2.70	0.000	0.000	39	35.03	0.000	0.000	60	454.69	0.000	0.000	81	5901.02	0.000	100.000
19	3.05	0.000	0.000	40	39.58	0.000	0.000	61	513.71	0.000	0.000	82	6667.10	0.000	100.000
20	3.45	0.000	0.000	41	44.72	0.000	0.000	62	580.41	0.000	0.000	83	7532.65	0.000	100.000
21	3.89	0.000	0.000	42	50.53	0.000	0.000	63	655.76	0.000	0.000	84	8510.56	0.000	100.000

Particle Size Report of Silver nanoparticle PXN

7.7 Zeta Potential Analysis

S.No	Sample	Zeta Potential (mV)	Stability behavior of sample
1	**Paclitaxel**	-57.3	Good stability

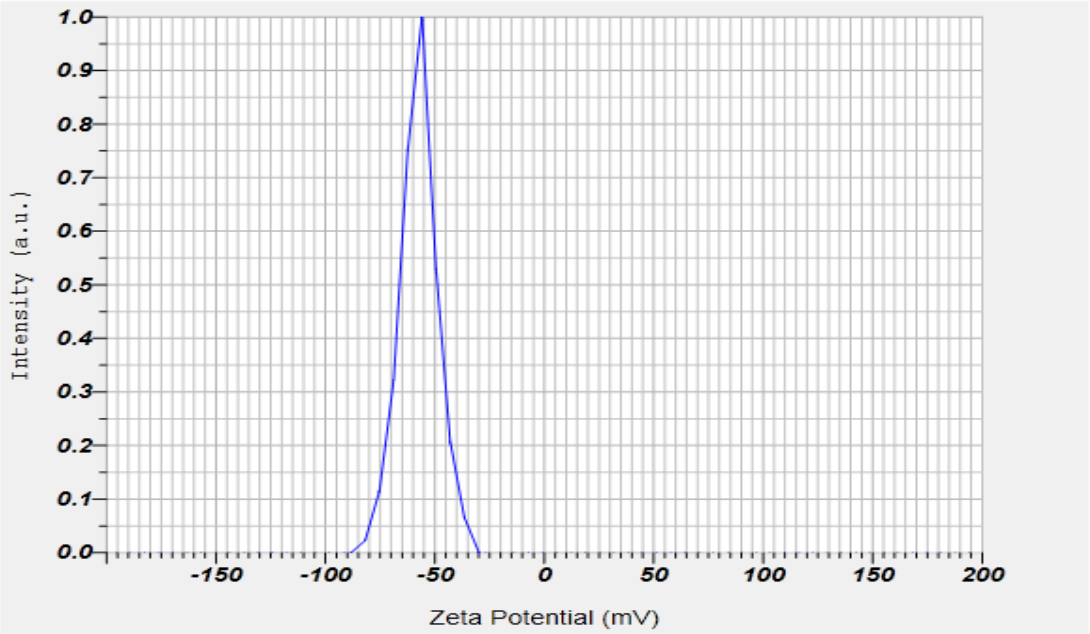


Fig.43:Zeta potential Analysis of Paclitaxel silver nitrate nanparticles.

PaclitaxelNanoParticals-Zeta.nzt

Measurement Results

Date	:	18 April 2017 16:21:19
Measurement Type	:	Zeta Potential
Sample Name	:	PaclitaxelNanoParticals-Zeta
Temperature of the holder	:	25.0 deg. C
Viscosity of the dispersion medium	:	0.894 mPa.s
Conductivity	:	0.096 mS/cm
Electrode Voltage	:	3.9 V

Calculation Results

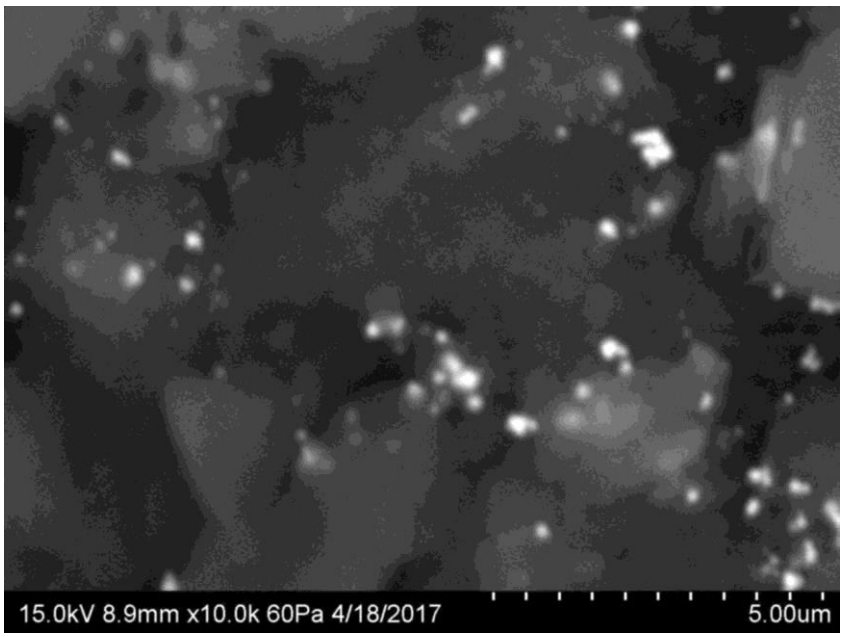
Peak No.	Zeta Potential	Electrophoretic Mobility
1	-57.3 mV	-0.000445 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-57.3 mV
Electrophoretic Mobility mean	:	-0.000445 cm2/Vs

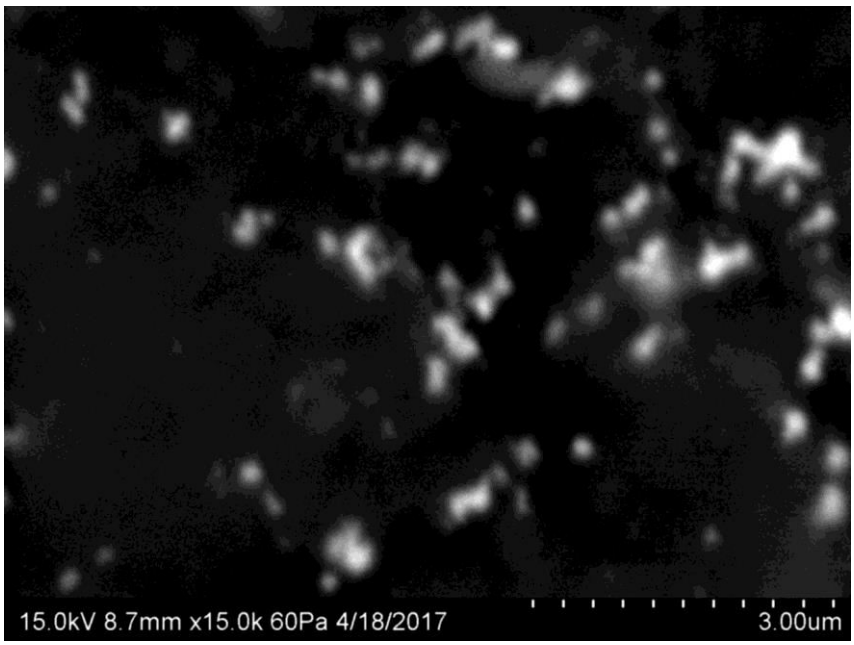
Zeta Potential Report of Silver nanoparticle of paclitaxel

7.8 SEM Analysis:

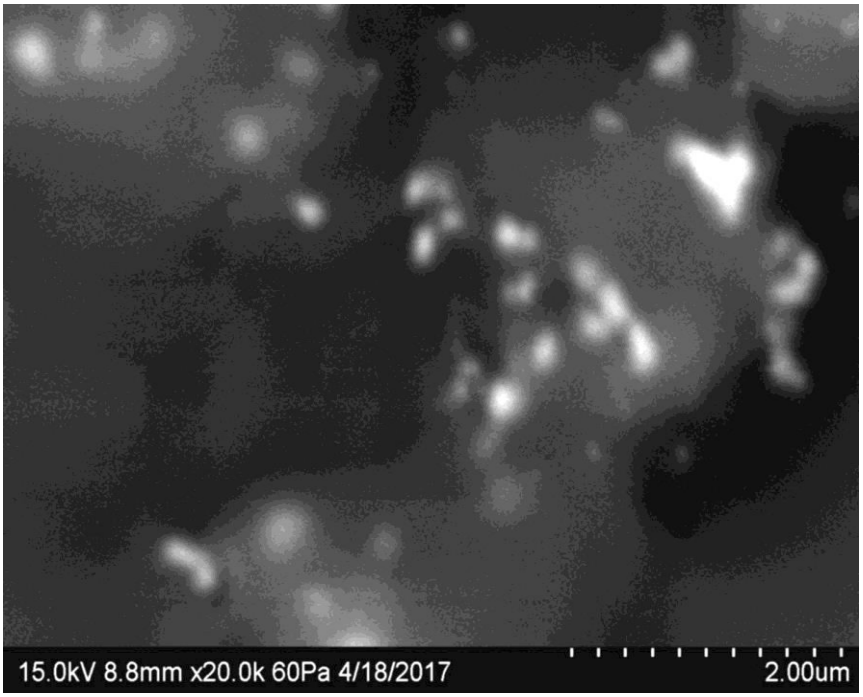
Fig.44: SEM images of Loaded Nanoparticles for best formulation PXN- 6



(a- M 10,000)



(b M- 15,000)



(c M-20,000)

7.9 Acceleratd stability study:

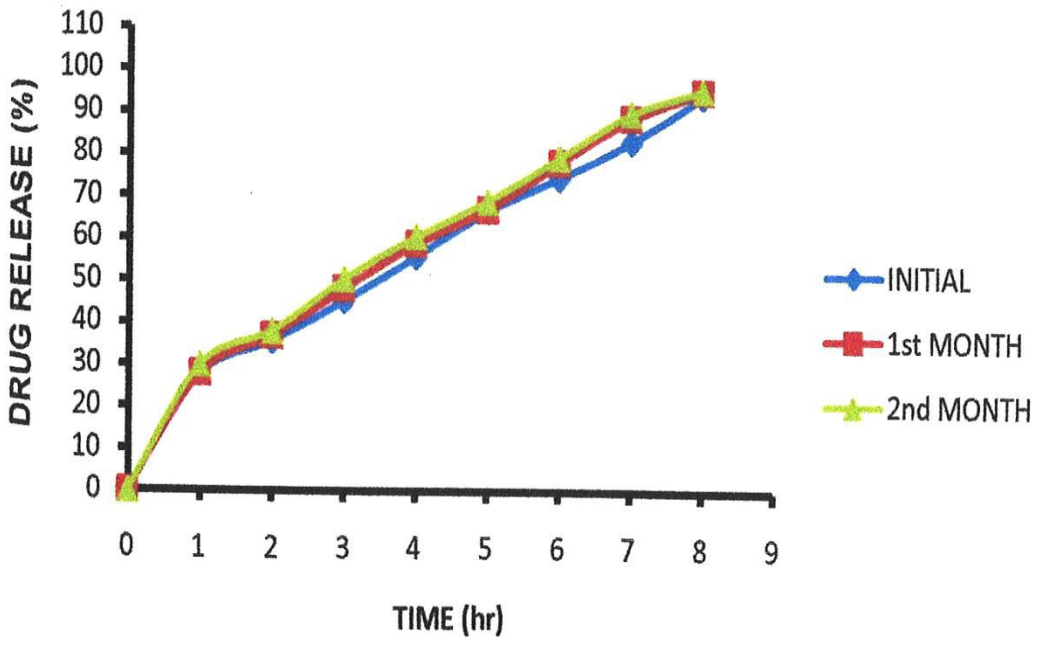
Table 31 : Stability testing parameters for optimized Paclitaxel silver nitrate Nannoparticles

Parameter	Storate condition 40°C ± 2°C & 75% ± 5% RH		
	Initial	**1st Month**	**2nd Month**
Appearance	Light yellow	Light yellow	Light yellow
Average weight (mg)	350mg	350mg	350mg
Drug content(%)	99.1	99.0	98.54

Table 32 *: In-vitro* dissolution study of Nanocapsule

Dissolution Time (hr)	Storate condition 40°C ± 2°C & 75% ± 5% RH		
	Initial	**1st Month**	**2nd Month**
1	27.69	27.94	29.98
2	35.53	36.86	38.014
3	45.23	48.12	50.46
4	55.84	59.01	60.69
5	66.92	67.13	69.14
6	74.76	78.45	79.69
7	83.53	89.16	90.45
8	94.61	95.63	96.12
9	96.51	96.53	96.55
10	97.61	97.63	97.70
12	97.81	97.85	97.88
24	98.61	98.63	98.67

Fig.45: Comparative dissolution profile of Paclitaxel silver nitrate Nannoparticles (stability study)



Discussion

8.1.1 Selection of Drug and Excipients:

Formulation development started from selection of API, the cost efficiency, easy availability and challenging aspects of drug properties made to select Paclitaxel. Then the excipients were selected based on the previous studies and compatibility.

8.1.2 Selection of Method:

Preparation of Nanoparticles started from the selection of suitable methodto prepare Nanoparticles. The Silver nitrate and Trisodium citrate was selected and individual Nanoparticles were prepared and evaluated. Silver nitrate and trisodium citrate were used as starting materials for the preparation of Paclitaxel silver nitrate Nanoparticles. The silver colloid was prepared by using chemical Precipitation method. This was achieved after a many trail batch. Finally the formulation of Paclitaxel Nanoparticle was finalized.

8.2 Raw material analysis of Paclitaxel:

8.2.1 Description and solubility:

The description of the Active Pharmaceutical Ingredient of Paclitaxelwas found to be complies with USP. Solubility of the Paclitaxel was found with different solvents. The results were shown in **Table17** and it was freely soluble in Methanol, Dichloromethane, Ethanol, Slightly soluble in Octanol, Ethylacetate and poorly soluble in Water.

8.2.2 Identification test:

The test Absorption time ,Melting point, Retention time and assay was done to identify the Paclitaxel .The results were shown in **Table 13& 14** that all parameters were complies with USP.The percentage purity of Paclitaxel was not less than 97.0% and not more than 102.0% calculated on the anhydrous , solvent-free basis.

8.3 Incompatibility study:

8.3.1 Fourier Transformer Infrared spectroscopy (FT-IR):

The development of a successful formulation depends only on a suitable selection of excipients. Hence the physical state of the drug paclitaxel, silver nitrate

and the combination of drug and polymers used for Nanoparticles preparation were studied by FTIR (Fourier transform infrared spectroscopy) to know the drug – polymer compatibility.The physicochemical compatibility of the drugs and the polymer was obtained by FTIR studies. The interpretation values of the FTIR were mentioned in the **Table 17**.Therefore, there was no alteration and no interaction was observed between polymer and drug in combination. All the characteristic peaks of, were present in combination, thus indicating no compatibility between drug and polymers and finally confirm that there was no chemical modification of drug have been taken place.Thus, from IR spectra studies we can draw a conclusionthat the drug remains in its normal form without undergoing any interaction with the polymers.

8.4 Evaluation of Paclitaxel Nanoparticles:

8.4.1 Nanoparticles size determination:

The particle size of the prepared Nanoparticles was determined by particle size analyzer (Beckman Coulter) and it was shown in **Fig. 42**. The Average particle size of the loaded Nanoparticles were found to be 717.4 ± nm. All the, prepared formulations were found to be in the nano range.

8.5 Pre-formulation Evaluation:

The angle of repose for the formulation was determined and was found to be within the limit .The formulation PXN-6 had excellent flow and other formulation showed good flow.

The bulk density of all formulations was found to be in the limit of 0.4 to 0.6 hence it was within the limit.

The Carr's index and Hansuer's ratio were found to be within the limit the results were shown in **Table20**.

8.6 Drug release kinetics:

The mechanism of drug release can be predicted by 'n' value:

- If 'n' values which is less than 0.45, the drug release mechanism would be Fickian diffusion mechanism.
- If 'n' value is more than 0.45 and less than 1, the release mechanism would be non- Fickian diffusion.
- If 'n' value is equal to 1, the drug release mechanism would be case II transport (Zero order release).

- If ‘n’ value is more than 1, the release mechanism would be super case II transport.

All the prepared formulations are having diffusion exponent value (n) more than 0.45 and less than 1, this indicates that the release mechanism follows non – Fickiandiffusion. The release kinetic graphs for best formulations are shown in **Fig**.**39 to 41**.The linear regression analysis of Nanoparticles shown as R2 values in **Table 29**. When the data were plotted according to the zero-order equation, for all formulations (PXN-1 to PXN-6) showed a fairly linear, with regression (R^2) values between (0.869 to 0.935) clearly indicate that the drug wasn't released as per zero order mechanism. All the formulation expressed by first-order plots shows linearity with regression coefficient (R^2) value as (0.953 to 0.981) also not close to infinity indicate the drug release process is not as per First-order plot. The Higuchis plots of all formulations were found to be highly linear, and close to infinity as indicated by their high regression (R^2) values as (0.98 to 0.993). Therefore, it was ascertained that the drug permeation from these formulations could follow either near Higuchis or Higuchis order kinetics. Hence the release mechanism was shifted from the zero order to first order, followed by Higuchis release kinetics.

Further, to understand the drug release mechanism, the data were fitted to Pappas equation. In the present study also it was observed **Table 27** that no value was obtained between (0.686 to 0.918) for all formulations. These values, suggesting that more than one mechanism may be involved in release kinetics.

8.7 Entrapment Efficiency:

As seen from drug loading, Encapsulation Efficiency of Paclitaxel NP was high (above 90%) for formulation prepared with TSC. However, it increased from 89.78% to 96.66% with use of TSC as shown in **Table 4**. This could be due to the fact that is due to the high solubility of drug in solvent resulting in high concentrations of the drug present in the preparation medium in the precipitation method.

8.8 *Invitro* dissolution study

In vitro drug release from the Paclitaxel NP in phosphate buffer pH 7.4 was performed using dialysis bag diffusion technique. The in *vitro* drug release profile of Paclitaxel NP formulations obtained from dialysis experiment is shown in **Fig.10** No burst release of paclitaxel NP was observed from these any of the eight formulations but formulation PXN-1 and PXN-8 gave 12 % of the initial drug release might be due

to lower silver nitrate concentration (0.001M) and lower concentration of TSC(3 ml), increases drug release slightly. Paclitaxel is a hydrophobic drug and thus it is expected that it will remain in hydrophobic domains of the precipitate. Entire drug release profile of paclitaxel was seemed to be diffusion-controlled as reported.[67]

It was observed that the drug release from the formulations slightly decreases as the particle size of the formulation decreases and all the eight formulations showed a continuous drug release from first hour. But upon increasing the concentration TSC cross linking agent by double, release was found to be decreases from beginning. This could be viewed as reduction of silver by citrate concentration yielded more hydrophobic surface which retard drug release. The drug release found to be dependent on the concentration of both silver nitrate and TSC material employed. Nanoparticles prepared with high concentration of Silver nitrate showed only 88% (PXN -8) of drug release at 24 hour. When silver nitrate concentration was at low (PXN 1-PXN6), release was almost by 90% at 24 hour. Release mechanism model was chosen, based on goodness of fit test. Based on the highest regression values (r), the best of fit model for all six formulations was Higuchi Model. Peppa's model with n ~ 0.5 value indicating non-fickian diffusion release. Thus showing release of PXN-6 was found to follow Higuchi's classical diffusion model and diffusion was non-Fickian

8.9 Optimization:

The effect of Trisodium citrate (TSC- cross-linking agents) on the characteristics of the Paclitaxel Nanoparticle (NP) prepared was studied. The Paclitaxel NP were characterized for entrapment efficiency, particle size, *in-vitro* drug release, and surface morphology. The TSC cross-linked Paclitaxel NP was white in color and was obtained as a fine powder. As shown in Table 9, the TSC volume used for cross-linking does have influence on the entrapment efficiency and the particle size of the Paclitaxel NP. The Paclitaxel NP cross-linked using TSC shows high entrapment efficiency of around 96.6%. The average mean diameters of the TSC cross –linked Paclitaxel NP ranged from 1.51 μm to 635 nm.

As shown in Figure 45, an increase in the concentration of TSC led to a decrease in the rate of drug release. Paclitaxel NP prepared using 3.0 ml of TSC releases the drug slowly compared to the Paclitaxel NP in which 10 ml of TSC was used. However, a burst effect was not observed in any of the formulations. In general, around 50% of

the drug is released in the 7-8 hour, followed by slower release for a period of 24 h. From fourth hour, the drug release was controlled manner for a period of 12 h. There is a difference in the drug release rates of the all formulations. The scanning electron microscopy study reveals that the surface of the microspheres is rough which may be because of the drug present on the surface.Based on particle size, Entrapment efficiency and release rate, Formulation PXN-6 was optimized for Further study.

8.9.1 Zeta Potential:

Measurements of zeta potential were also carried out in order to study the stability of nanoparticles as this extremely important for many applications, Surface zeta potentials were measured using the zeta analyzer (Beckman Coulter Delsa Nano C, Brea, USA) Liquid samples of the nanoparticles (5ml) were diluted with double distilled water (30 mL) and the pH was then adjusted to the required value. The samples were shaken for 30 minutes. After shaking the zeta potential of the metallic particles was measured. A zeta potential was used to determine the surface potential of the silver nanoparticles. In each method, an average of two separate measurements was reported. **Table 31** is summarizing the zeta potential measurements of samples in a solution form. For synthesis nanoparticles using different methods, zeta values were measured and found to be – 57.3 mV at pH=7. The value of the zeta potential of Silver NPs provides satisfactory evidence about their little tendency towards aggregation when its negative charges with a diameter of 717.6 nm. This behavior unambiguously suggests the presence of strong electric charges on the particle surfaces to hinder agglomeration. These values were found to fall in the negative side which showed the efficiency of the capping materials in stabilizing the nanoparticles by providing intensive negative charges that keep all the particles away from each other. This result suggests that the Silver NPs particles and thus their solution is stable behavior a shown in **Fig. 43.**

.

8.9.2 Scanning Electron Microscopy

SEM analysis was performed on the prepared loaded Nanoparticles to access their surface morphological characteristics of prepared Silver NPs using Tri sodium citrate a)Low, Medium and b) high magnifications as shown in **Fig. 44 (a.b.c).**

SEM was performed for best formulations to assess their surface. The polymer surface of the Nanoparticles appeared spherical with smooth texture surface. Discrete nature, and distinct particle size and shape with a smooth surface.

The morphology particles were measured. The silver Nanoparticles in the citrate cross linked were in a spherical form with a well defined particle size. The particle size strongly depend preparation conditions. The average particle size of the measured particles was as small as 645 nm to 1.12 µm.

8.9.3 Stability study:

The colour and shape of Paclitaxel were found to be unchanged even at the end of 2nd month stability study in all conditions. The results were shown in **Table 32 to 33&Fig45**.In order to perform the stability study the Nanoparticles were placed with blister packing material and folded in to the strips, then placed into stability chamber .At the end of the month one set of the colloidal Nanoparticle were analyzed for shape average weight and drug content. There was no change in the colour and shape of Nanoparticles .Also no changed absorved in release behavior up to two months when compared to optimized formulation. Sufficient precautionary measures were taken to prevent the photolytic degradation of Paclitaxel.

Summary

Drug delivery systems to the colon are being actively investigated in order to develop oral preparations of peptides and treat local colonic diseases, e.g., irritable bowel syndrome, ulcerative colitis, cancer, and infection.Paclitaxel is a microtubule-stabilizing agent which promotes polymerization of tubululin causing cell death by disrupting the dynamics necessary for cell division. It has neoplastic activity especially against primary epithelial ovarian carcinoma, breast, colon, and non-small cell lung cancers.

From the present investigation, the obtained results were summarized as follows;

- The IR spectrum of pure drug and drug mixture indicated that there was no interaction between polymers and drug.
- The Nanoparticles prepared were small, spherical with smooth surface having better yield and uniform encapsulation efficiency.
- Paclitaxel nanoparticles were having an average particle size of 717.4 ± nm.
- The Zeta Potential analysis showed the stability of colloidal dispersions of Nanosparticles. The Paclitaxel Nanoparticles showed with excellent stability of
- -57.3mv
- The shape of Nanoparticles was almost spherical and smooth as indicated by SEM.
- Encapsulation efficiency for Paclitaxel nanoparticles was in the range of 73.89% to 95.9% as the polymer concentration increases, the encapsulation efficiency also increases
- Paclitaxel release from Nanoparticles was slow and extended period of time due to increase in polymer concentration. The release mechanisms for all the formulations followed by non-fickian diffusion mechanism.
- The drug release from formulation PXN6 was 92.85% for a long period of 24hrs. The release mechanisms for all the formulations followed by non-fickian diffusion mechanism.

  Hence Nanoparticles prepared by precipitation method using silver nitrate showed promising results in delivering the drug, and there exist a scope for *in-vivo* evaluation using suitable animal models.

Conclusion

The present *in vitro* study revealed that the Paclitaxel NP can be a useful means for the colon targeted delivery of drugs. Around 60% of the drug to be released at colon after regular transit (GIT) of 4-6 hour. This work suggests the Paclitaxel NP of the size ranging from 635 nm -717 nm and their solutions are stable at neutral pH. The prepared Nanoparticle found to be stable without any tendency of aggregation and shown higher entrapment efficiency. The hydrophobic surface of the drug with silver release the drug slowly but up to 24 hours only. Because of higher in size of prepared Nanoparticle, release couldnot be prolonged for more than 24 hours: this finding is considered as drawback. The dissolution data indicates that the release of Paclitaxel NP with controlled manner is directly proportional with the size and concentration of Silver nitrate and crosslinking agent. Therefore, nanoparticles release increased with smaller size of particles. In this study, the prepared paclitaxel NP exhibited prolonged intestinal absorption, and prevent gastric release, avoid gastric erosion side effects and thus improve patient compliance. Short term stability study reveal formulation in Capsule dosage form found to be stable without any major problem. However, further studies are needed to investigate these formulations to prepare Nanoparticles with desired size range of around 200 nm.

Bibliography

1. Vyas SP, Khar RK Targeted & Controlled Drug Delivery Novel Carrier System. CBC Publication 2002; 6(2):249-227,331-387.

2. Majeti NV, Ravi Kumar A review of chitin and chitosan applications. Reactive and Functional Polymers 2000; 46(1):1-27.

3. Katija J, Wolfgang M,Markus D,Heike B. Investigations on the structure of solid lipid nanoparties (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy,field-flow fractionation and transmission electron microscopy. Journal of Controlled Release 2004; 95(2):217-227.

4. Kreuter J. Nanoparticulate systems for brain delivery of drugs. Adv Drug Deliv Rev March 2001; 47(1): 65-81.

5. Ulrike S, Bernhard A, Sabel, Helmut S. Diffusion enhancement of drugs by loaded nanoparticles. Neuro-Psychopharmacol & Bio-Psychiat 1999; 23(12):941-949.

6. Zhang DY,Shen XY,Wang JY,Dong L,Zheng YL,Wu LL. Preparation of chitosan poly aspartic acid -5-fluorouracil nanoparticles and its anti-carcinoma effect on tumor growth in nude mice. World J Gastroenterol 2008; 14(3):3554-3562.

7. Arias JL. Novel strategies to improve the anticancer action of 5-fluorouracil by using drug delivery systems. Molecules 2008; 13: 2340-2369.

8. Duran JDG,Arias JL,Gallardo V,Delgado AV. Magnetic colloids as drug vehicles J Pharm Sci .2008;97:2948-2983.

9. Wong HL, BendayanR, Rauth AM, Li Y,Wu XY. Chemotherapy with anticancer drugs encapsulated in solid lipid Nanoparticles .Adv Drug Deliv Rev 2007; 46: 149-168.

10. Jain RK. Delivery of molecular and cellular medicine to solid tumors. Adv Drug Deliv Rev 2001; 46: 1231-1242.

11. Reddy LH. Drug delivery to tumors recent strategies .J Pharm Pharmacol 2005; 57: 1231-1242.

12. Davis ME, Chen Z, Shin DM. Nanoparticles therapeutics an emerging treatment modality for cancer. Nat Rev Drug Discov 2008; 7: 771-782.

13. Arias JL,Gallardo V,Gomez-Lopera SA,Plaza RC,Delgado AV. Synthesis and characterization poly(ethyl-2-cyanoacrylate) Nanoparticles with a magnetic core . J Control.Release 2001; 77: 309-321.

14. Brigger, Dubernet, Couvreur. Nanoparticles in cancer therapy and dignosis. Adv Drug Deliv Rev 2002; 54: 631-651.

15. Shina VR, Bansal K,Kaushik R,Kumaria R,Trehan A. Saynthesis and charecterization of poly-caprolactone nanoparticles with magnetic core. IntJ Pharm 2009; 17(3): 289-357.

16. Muller RH, BohmB. A formulations approach for poorly soluble and bioavailable drugs. In Dl Wise, Editor, Handbook of pharmaceutical released technology 2002; 45-357.

17. Pignatello R, Bucolo C. Nanosuspentions for the ophthalmic controlled delivery of ibuprofen. Eur J Sci 2004; 16(1-2): 53-61.

18. Liversidge GC. Drug nanocrystals for improved drug delivery Int Symp Control Release Bioact Matter 1996; 23(): 30-35.

19. Akerman ME, Chan WC. Nanocrystals targeting *in vivo*. Proc Natl Acd Sci USA 2007; 99: 12617-12935.

20. Hishiyama N, Kataoka K. Polymeric micelle drug carrier systems and second generation of micellar drugs. Adv Drug Delivery Rev2003; 519: 155-177.

21. Nagasaki Y. Block copolymer micelles for drug delivery: Design, Characterization and Biological significance. Adv Drug Delivery Rev. 2001; 47: 113-15.

22. Vayas SP and Malaiya A. Microencap. 1989; 6: 493.

23. Ayutlede J, Gandhi M. Carbon nanotube reinforced bombyx morisilk nanofibres by the electrospining process. Bioacromolecules2006; 7: 208-14.

24. Allet TM. Liposomes: Opportunities in drug delivery. Drugs 1997; 57: 8-14.

25. Vyas SP and Malaiya A.In-vivo characterization of indomethacin magnetic polymethyl methacrylate nanoparticles Microencap. JPharmSci 1989; 6(4) 493-9.

26. Babincova M, Babinnec P. High gradient magnetic capture of ferrofluids: Implication for drug targeting and tumor immobilization.Z.Naturforsh 2001; 56: 11-909.

27. Katoka K, Harada. Nanoelectronics. Nano Lett 2001; 519: 155-177.

28. Jain NK. Controlled & novel drug delivery 1st edition. CBS Publication 2001; 85:292-301.

29. VJ Mohanraj & Chen Y. Nanoparticles – A review. Tropical Journal of Pharmaceutical Research 2006; 5(1): 561-573.

30. Mamun UR,Md KHB, Emran Q. Syenthesis of silver nanoparticles (Ag-NPs) and their uses for quantitative analysis of vitamin C tablets. Dhaka Univ J Pharm Sci 2013; 12(1): 29-

31. Yujie W, Guoqiang, Tingt IQ & Fuxin D. Preparation and evaluation of paclitaxel-loaded nanoparticle incoporated with galactose carrying polymer for hepatocyte targeted delivery. Journal Drug Development & Industrial Pharmacy 2012; 38(9): 523-635.

32. Nagoc DT, T TBN &Thanh HN. Preparation and characterization of silver chloride Nanoparticles as an antibacterial agent .Adv Nat Sci Nanosci, Nanotechnol 2015; 6: 65-81.

33. Dinda,I Biswal, P Chowdhury,R.Mophapatra. Formulation and evaluation of paclitaxel loaded solid lipid nanoparticles using glyceryl monostearate. Journal of Applied Pharmaceutical Science 2013; 3(8): 133-138.

34. Shuben S, Ping L, Faming S. Formulation and evaluation of PLGA Nanoparticles loded capcitabine for prostate cancer. Int J Clin Exp Med. 2015; 8(10): 19670-19681.

35. Natrajan T, Chellan VR. Formualation and characterization of anti-Alzheimer drug loaded chitosan nanoparticles. Journal of Young Pharmacist 2015; 17(1): 232-536.

36. CA Anuradha and Jithan A. Charecterization and In vivo evaluation of Bis-demethoxy curcumin anlouge (BDMCA) nanoparticles. Tropical Journal of Pharmaceutical Research 2010; 9(1): 51-5820.

37. Jiaojio X, Behihua, Dan Shou, Xiaojing X and Ying H. Preparation and evaluation of cefixime Nanoparticles prepared using fenugreek seed mucilage and chitosan as natural polymer. Int Pharm 2015; 7(1): 1850-1870.

38. Shanmuganathan S, Harika B, Gowthamrajan k. Preparation and evaluation of cefixime nanoparticles. In J Pharm 2016; 8(3): 179-188.

39. Yu-Ling F,Bing-Yu F,Qiang L,Hai-Xiao D ,Xiang-Yu M,Na L. .Prepartion of 5-fluouracil loaded nanoparticles and study of interction with gastric cancer cells 2015; 15(18): 7611-7615.

40. Shanshak T, MN Satish K and Ashwati P. Formulation and in vitro characterization of carbamazepine polymeric nanoparticles.J Chemical Pharm 2015; 7(2): 70-79.

41. Pragati L, Ganesh Kb, Preeti K. Formulation and evaluation of ampicilin loaded nanoparticles. ISJPS 2015; 5(1): 1289-1297.

42. Vijayanthimala T, Bharathi DR, Lackshmi RG, Mallamma T, Dinesh R. Formulation and evaluation of zidovudine loaded chitosan nanoparticles. International Journal of Biopharmaceutics 2014; 5(1):512-528.

43. Shumaia P,Md Abu SR,MD Abdul K. Formulation and evaluation of dexmethasone loaded stearic acid nanoparticles by hot homogenization method. International Current Pharmaceutical Journal 2014; 3(12): 331-335.

44. Anand KK, Parameswara RV, Priyanka K, Sanjay KS, Sanjay S. Development and evaluation of solid lipid nanoparticles of raloxifene hydrochloride for enhanced bioavailability. Bio Med Reseach International 2013; 52(2): 1-9.

45. Riddhi DKP, Mitali S, Hiral M, Jigna A, Shweta B. Preparation and *In vivo* study of doxorubicin HCL loaded chitosan nanoparticles prepared by w/o emulsion method. 2012; 4(12): 438-440.

46. Can Z, Yao C, Guowei Q, Xiaoli W, Ya D, Zhihong C, Liangli Y, Qineng P. Preparation and characterization of galactosylated chitosan coated BSA microspheres containing 5-fluoruacil carbohydrate polymers. 2008; 72(3): 390-397.

47. C.Zinuttia, F Kedzierewicza, M Hoffmana & P Maincenta. Preparation and characterization of ethyl cellulose microspheres containing 5-fluoruacil. 1994; 11(5):302-329.

48. Official from May 2012. Copyright (c) 2011 the United States Pharmacopoeial Convention.

49. Rxlist.com http://www.rxlist.com/zofran-drug.htm (Acessed 15th may 2017).

50. Drug bank (17-03-2015,12:12:27PM) Paclitaxel available at http://www.drugbank.ca/drugs.(Acessed:3rd July 2017).

51. Wadke PA, Jacobson S. Preformulation testing In: Liberman HA and Lachman L. editor's pharmaceutical dosage forms- Tablets, New York, Marcel Dekkar Inc. 1980; 1:1.

52. Raymond C Rowe, Paul J Sheskey and Sian C Owen .Handbook of pharmaceutical excipients, fifth edition; PP 278-282.

53. Zhang C, Ping Q, Zhang H, Shen J. Synthesis and characterization of water-soluble O-succinylchitosan. Eur Polym J**.** 2003; 39:1629–1634.

54. Indian Pharmacopoeia, Government of India. Ministry of Health and Family Welfare, Controller of publication, New Delhi.1996; 1: 423-424.

55. Lee M, Cho Y and Chung H. Size control of self-assembled nanoparticles by an emulsion/solvent evaporation method. Colloid Polym Sci 2006; 284: 506-512.

56. Quintanar, GD, Ganem, QA, Allemann E and Fessi H. Influence of the stabilizer coating layer on the purification and freeze drying of poly (DL-lactic

acid) nanoparticles prepared by emulsification-diffusion technique. J Microencapsualtion 1998; 15: 107-119.

57. Teraj M, Sunil A. Novel interpenetrating network chitosan poly (ethylene oxide-g-acrylamide) hydrogel microspheres for the controlled release of capecitabine. Int J Pharm 2006; 32: 103-115.

58. Donbrow M, Samuelov Y. Zero order drug delivery from double-layered porous films: Release rate profiles from ethyl cellulose, hydroxy propyl cellulose and polyethylene glycol mixtures. J Pharm Pharmacol. 1980; 32: 463-470.

59. Sandri. Chitosan-associated SLN in vitro and in vivo characterization of cyclosporine a loaded ophthalmic systems. J Microencapsul. 2010; 27: 735-746.

60. Gadha A, Raina HF. Diazepam loaded solid lipid nanoparticles design and characterization.AAPS pharma Sci Tech. 2009; 10(1):221-19.

61. Freitas M.N, Marchetti J.M. Int J Pharm. 2005; 295: 201.

62. Libo Y, Reza F. J. Pharm. Sci. 1996; 85: 170.

63. Narashimhan B, Mallapragada SK, Peppas NA, Mathiowitz EED, John W and Sons. Releasekinetics data interpretation in Encyclopedia of controlled drug delivery.Inc, New York 1999.

64. Silvina A, Bravo M, Lamas C, Claudio J.In-vitro studies of diclofenac sodium controlled-release from biopolymeric hydrophilic matrices. J Pharm. Sci. 2002; 5(3): 213-9.

65. Grassi M, Grassi G. Mathematical modeling and controlled drug delivery matrix systems. Curr Drug Deliv. 2005; 2: 97-116

66. Higuchi T. Mechanism of sustained action medication, theoretical analysis rate of solid drugs dispersed in solid-matrices. J Pharm Sci. 1963; 52:1145-1149.

67. Peppas LB, James OB, Nanoparticle and targeted systems for cancer therapy. Adv Drug Del Rev, 56 (2004) 1649– 1659.